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Mechanisms of Density-Dependent Population Regulation in the Marine Copepod *Amphiascoides* sp. (Harpacticoida)*

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ABSTRACT: *Amphiascoides* sp. was cultured in the laboratory for the first time. Culture method and data on its life cycle are given. Reproductive potential and behaviour (pairing incidence) were studied in relation to population density (i.e. to medium conditioned by a previously dense population) and in relation to food supply. It was found that: (1) conditioned medium reduces the number of ovisacs per female by reducing its fertile period and by extending the interval between release of successive pairs of ovisacs, older females being more susceptible to this inhibition; (2) conditioned medium causes partial sterilization (non-fertilization?) of eggs; (3) these effects are partly, but not completely, reversible when females are returned to fresh medium (delayed, density-dependent effects); (4) crowding itself, but not medium conditioned by crowding, reduces the number of eggs per ovisac; (5) ovisacs are not released in absence of food, and food supply triggers the release of ovisacs; (6) mating incidence is a function of food supply; (7) mating incidence is higher in groups than in isolated pairs; (8) repeated mating is necessary for sustained fertility; (9) infertile periods in females as a result of deprivation of food or males may be compensated for by extension of the normal (50–60 d) fertile period to maximally 80–100 d; (10) the generation period (nauplius to fertile adult) increased by 40 % in the course of laboratory existence. These mechanisms of true population regulation via negative feedback are discussed in the light of the 'paradox of evolution under competition': resources are limited, yet, those genotypes that are getting more numerous than others (higher fitness) win the competition (= positive feedback).

INTRODUCTION

As earlier studies on population regulation in various invertebrates (Walker, 1967, 1975a, b; Walker and Williams, 1976), the present investigation results from the interest in the physical aspects of evolution. Under conditions of competition, that is when carrying capacity of a given factor is reached, a stable population has a growth rate of $r = 0$ (Malthusian fitness), when each dying female is replaced by a single fertile daughter (Whitian fitness, $f = 1$). In order to replace this population by a better genotype, the fitness of the new genotype would have to be larger than the one of the original genotype ($r' > 0$; $f' > 1$). Hence, the population would grow hyperbolically as an ever increasing portion of individuals propagates with the fitness r' until all individuals are replaced by the new genotype. Yet, resources for this growth are not available, since the original population already operated at carrying capacity.

Evolution under competition thus represents the classical positive feedback, where the discrepancy between the 'sollwert' (population growth = zero) and the actual value is steadiliy increasing. Such populations fall into pathological oscillations (Wangersky and Cunningham, 1957; Walker, 1967) with absurd densities followed by mass decline after which few survivors build up the next cycle under conditions of surplus resources. Consequently, possible selection for a given genotype is periodically destroyed by random genetic drift. The conclusion is inevitable that orderly evolution under conditions of competition is possible only if the genetic increase in fitness is balanced by a reduction in phenotypic population fitness, while differential relative fitness between the genotypes is preserved. This necessitates continuous correction of reproduction and/or emigration in relation to space and resources via negative feedback mechanisms.

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Aquatic organisms offer the advantage that the two factors, density per space and density per food supply, can neatly be separated in the analysis. At high spatial population densities competition for food can never be excluded, even if the experimenter is under the impression that he provided surplus food; physical interference between individuals – such as jostling, fighting or perhaps nervous disturbance – may prevent certain individuals from spending enough time feeding. Regulation of density per area is probably effected by the excretion of pheromones. Thus, culture water previously densely populated can be filtered and reused to measure reproduction of single, well-fed individuals in such conditioned medium. The effect of food supply, on the other hand, can be assayed in fresh medium at low densities.

Earlier studies by these methods showed mutual inhibition of reproduction at high density and stimulation of reproduction at low density in the ciliates *Paramaecium* (Robertson, 1921), *Tetrahymena* (Stillwell, 1967) and *Keronopsis* (Walker, 1967) as well as in algae of the family Volvocaceae (Harris, 1971). Demonstration of similar population behaviour in a crustacean would add support to the hypothesis that density-dependent population regulation via negative feedback mechanisms is a general condition of living systems.

Amphiascoides sp. seemed to be the ideal experimental organism. It lives in the coastal waters of the Dar-es-Salaam area (Tanzania), apparently in great abundance, as it turned up regularly in the sea-water supply for the University's aquaria. It breeds profusely on small pieces of boiled vegetation, and laboratory populations survive indefinitely with a minimum of care. For a recent review on copepod cultivation consult 'Marine Ecology', Volume III: Kinne (1977).

The observations reported in this study fall into three phases which are separated by several years: preliminary observations were made in 1967 in Dar-es-Salaam, several experimental series were carried out in London in 1971; the major part, however, was done from 1975–1976 in London. Hence, these experiments preceded the recent publications on population dynamics and ecology in various copepod groups (Gaudy and Guérin, 1977; Corkett and McLaren, 1978; Parrish and Wilson, 1978; Zurlini et al., 1978). These papers and some earlier ones will therefore be considered together with the results under 'Discussion' of this study.

MATERIAL AND METHODS

Material

The harpacticid was identified as *Amphiascoides subdebilis* Willey by Dr. W. Scheibel and Prof. W. Noodt (Zoologisches Institut der Universität Kiel, Federal Republic of Germany) and as *Amphiascoides cf neglectus* (Norman and T. Scott) by Dr C. B. Coull (Belle Baruch Institute for Marine Biology, Columbia, S.C., USA). All three specialists feel that the taxonomy of this genus needs re-consideration and that this organism may be a new species.

Methods

The sea water supply for our aquaria, from which *Amphiascoides* sp. was isolated, and which also served for its cultures, came from the intertidal zone of Oyster Bay beach (Dar-es-Salaam) and of Mbegani beach (ca. 16 km from Dar-es-Salaam).

The culture medium was fresh sea water which was passed through a Whatman paper filter and sterilized by heating for 10 min to just below boiling point. This treatment proved to be sufficient (Walker, 1975a, b) to avoid contamination by micro-organisms.

Food was prepared by boiling pieces of lettuce leaves for 10 min in sea water and later supending them in cold, sterilized sea water. Glass Petri dishes (5 cm ∅ for 1–20 individuals) and crystallizing dishes of 50 and 100 ml for larger numbers of copepods were used for culture dishes. These were closed by glass covers in order to prevent evaporation. The culture dishes were washed and sterilized in distilled water and never came into contact with soap, detergents and chemicals.

Establishment of laboratory culture and routine maintenance. The culture was established in September 1966 with copepods from a single sea-water supply. In order to reassure myself that the harpacticids of later supplies were in fact the same species, as well as to obtain a reasonable genetic heterogeneity in the laboratory culture, I isolated in December 1967 single, juvenile individuals from the laboratory stock and from a recent sea-water supply, then paired immature partners from the two sources and later returned breeding females to the laboratory stock. Thus, either these mothers, or the males that fertilized them, originated from the new supply. With the exception of a few preliminary observations all data recorded in the following refer to this 'Laboratory Stock'. This stock was pure insofar as there was no contamination by algae or protozoans. Furthermore, the culture water remained clear and showed no signs of bacterial contamination (in exceptional cases of accidental infection the respective cultures were eliminated). However, the detritus produced by the harpacticids may contain its own, intestinal micro-organisms. Thus, it cannot be affirmed that the laboratory stock was sterile.

Maintenance. At approximately monthly intervals (depending on the density and condition of the culture) 50–100 individuals of all ages together with freshly released ovisacs were transferred by pipette into new culture dishes with 100 ml of fresh medium and circa 2 cm^2 of boiled lettuce. Within this period the culture may reach a density of 60–80 individuals per 1 ml. *Amphiascoides* works itself into the tissue of the leaf between epidermis and hypodermis and feeds on the parenchyma. New food was added whenever the former supply was near exhaustion. At weekly intervals the culture water was exchanged by mere decanting. The harpacticids stay preferably at the bottom of their glass dish; after vigorous stirring the light waste particles remain in suspension much longer than even eggs and nauplii. Thus, the old medium can be decanted and replaced by fresh. The level of the water was always marked in order to control evaporation in which case the level was restored by addition of distilled water. The cultures were kept at 23 ° ± 1 C° under natural night/day conditions.

Experimental cultures. Experimental animals were raised under low density conditions (50–100 per 100 ml medium) with surplus food if not otherwise indicated. Conditioned medium was prepared in order to test the effect of high density on single or small groups of experimental animals. To this end a high density stock culture was left for 7–10 days without water exchange. Then, its medium was decanted and filtered first through a Whatman papier filter and then through a sintered glass filter with ultra fine pores together with 1/3 of fresh medium. This purified and somewhat diluted old medium is referred to as 'conditioned medium'. In all tests, experimental and control animals came from the same culture, the conditioned medium from the same dish for all replicates, and the control medium consisted of fresh medium filtered by both methods as the conditioned medium. Conditioned medium and control medium came invariably from the same sea-water supply.

A-, B-, C-cultures. From May 1975 to January 1976 three different culture regimes were maintained in three isolated breeding stocks.

A-culture was kept at low density; in the extreme the density before renewal reached 15 ± 5 individuals of all ages per 1 ml, usually it stayed far below that; maintenance as described for the laboratory stock.

B-culture was kept at low density as A, but the culture water consisted invariably of conditioned medium; this was prepared from the 'Laboratory Stock' culture as described above. Routine maintenance as described for the laboratory stock.

C-culture density was not regulated and routine maintenance as described for laboratory stock; thus, the density oscillated between low and extreme values, and consequently, so did the condition of the medium.

RESULTS

Normal Development

A number of observations between 1966 and 1969 in Dar-es-Salaam (Table 1) showed that 4 moults at daily intervals brought the nauplius to the copepodite stage and that 6 further moults resulted in the adult. Deviations from this pattern were the exception and occurred more often during the later stages of development. Pairing and formation of ovisacs took place within 48 h after the last moult. The ovisacs were released within 1–3 d after their appearance and the nauplii hatched within 24 h. Copulation was invariably preceded by 'pair formation': the male attaches itself with its antennae to the tail furca of the female. Pairs remained joined for many hours. During this period repeated copulation took place, however, it is uncertain, whether each copulation was accompanied by renewed insemination. The sex ratio remained in the vicinity of 1 : 1 throughout. It was never determined by detailed counts, however, when raising a cohort of animals of equal age, one could rely on obtaining approximately

Table 1. *Amphiascoides* sp. Phases of development (range in days within which all individuals reach the respective stage)

Years and place	Total number of			Development (days after day zero)			
	Tests	Replicates	Individuals	Day zero	Meta-morphosis	Adult	First ovisacs
1966–1969 Dar-es-Salaam	8	22	300	Nauplius 1st stage	3–6	9–13	11–16
1971–1975 London	6	12	120	Nauplius 1st stage	6–9	14–19	18–28

half the number of breeding pairs. In a few cases longevity was determined: the mean life span was circa 4 months while the oldest individuals reached 160 days (Table 7). Pairing behaviour extended over the whole life span, whereas production of ovisacs ceased considerably earlier, depending on conditions of nutrition and fertilization (see pp. 215 and 216). New ovisacs were usually formed within 24 h after release of the former pair.

Later observations in London showed the curious fact that the generation period (nauplius to first reproduction) had increased by about 40 % (Table 1). One possible reason is the changed diurnal rhythm, long day/short day in London as against equatorial conditions in Dar-es-Salaam; however, this aspect was not investigated. The culture temperature was not involved as it was the same in both places.

Effects of Conditioned Medium on Production of Ovisacs

Data of three preliminary tests in Dar-es-Salaam (1969) showed that young, breeding females produced 46 % less ovisacs in conditioned medium than in fresh medium (38 experimental females, 42 controls; observation period = 8–15 days). The nature of this reduction was analysed in more detail in 1971 and 1975/76 in London.

Tests from 1971

Three test series were carried out with 10–12 experimental animals each and with the same number of controls. Young, mated females with their first ovisacs were isolated singly into 8 ml Petri dishes with medium and food. During 18 days, ovisacs formed and released were noted daily. The females were kept in well-fed condition throughout. The 31 controls produced a mean of 4.71 ± 1.22 pairs of ovisacs per female at average intervals of 2.37 ± 1.32 days, whereas the 31 experimental females in conditioned medium produced only 3.13 ± 1.28 pairs of sacs at intervals of 3.39 ± 1.41 days (*t*-test, $P_{ovs} < 0.05$; $P_{days} < 0.05$). The histograms from which these means are derived are shown in Figure 1. Conditioned medium has two separate effects: it extends the interval between the release of successive ovisacs and it reduces the number of ovisacs produced. As the observation was carried on until the experimental females had ceased to reproduce, the reduction of ovisacs per female is not the result of the extension of the period between successive pairs of ovisacs; the experimental females had enough time to produce whatever they were capable of.

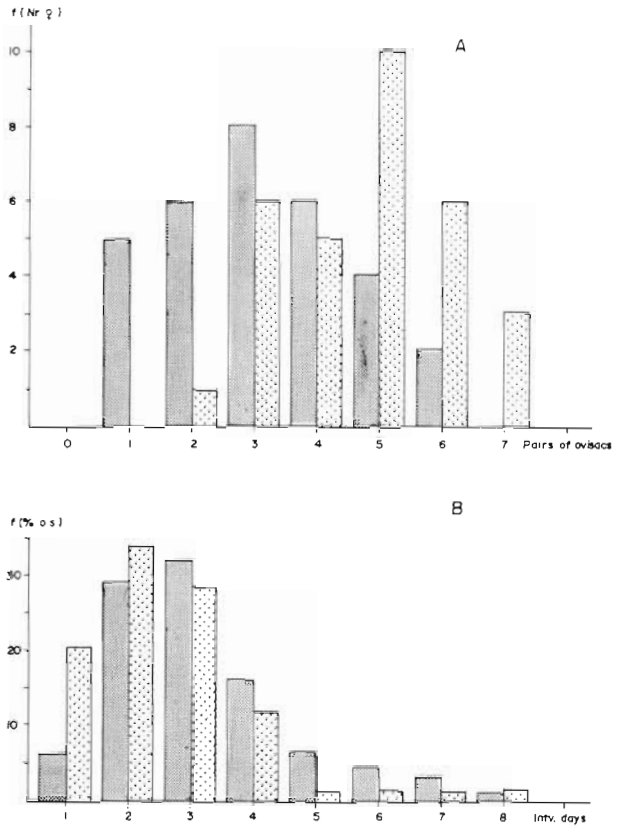

Fig. 1. *Amphiascoides* sp. Production of ovisacs in conditioned medium (31 experimental females, dark columns) and in fresh medium (31 control females, light columns). A: frequency of females [f (Nr. ♀)] producing various numbers of ovisacs. B: Percent ovisacs [f (% o. s.)] released at certain intervals (intv. days) between successive pairs of ovisacs

Considering only the first of the three tests: of 59 second and further pairs of ovisacs produced (by experimental and control females), 55 appeared within less than 24 h after the release of the former pair. Periods of more than one day between release of successive ovisacs are thus characterized by the presence of ovisacs with delayed release, and not by delayed oogenesis and absence of eggs. The data of the second and third tests show a similar pattern, but are not presented in detail.

Continuation of test 3: The females of the third test (Sept. 1971) were subject to further observation; 9 experimental and 10 control females lived sufficiently long so that the end of their reproductive period could be ascertained. After the initial 18 days of observation test and control females were divided into two groups each, one transferred into fresh medium and one into conditioned medium. Males were added to allow for renewed mating, and the harpacticids were kept in well-fed condition over their whole life span. Reproductive period and production of ovisacs are shown in

Table 2. *Amphiascoides* sp. Reproduction in fresh and conditioned medium, 1971

	Medium:	Conditioned		Fresh	
Early period	Females tested (fertilized, 2–4 d old)	9 (C_1)		10 (F_1)	
	Observation period (days)	18 d		18 d	
	Ovisacs per ♀ and early period	6.4 ± 1.0		8.2 ± 1.1	
Later period	**Medium:**	**Conditioned**	**Fresh**	**Conditioned**	**Fresh**
	Females from above (♂ added)	5 (C_1C_2)	4 (C_1F_2)	4 (F_1C_2)	5 (F_1F_2)
	Last ovisacs after the number of further days given	14 d	42 d	33 d	63 d
	Ovisacs per ♀ and later period	0.6 (sterile)	8.5	4.8	12.6

Table 2. The results are summarized as follows: (1) Conditioned medium has an immediate repressive effect on the production of ovisacs. (2) Conditioned medium has a permanent effect on the production of ovisacs and on the reproductive period: conditioned, repressed females, when returned to fresh medium, do not recover their full reproductive potential. (3) The later phases of reproduction (i.e. older females) are much more susceptible to the inhibition by conditioned medium.

Developmental period: The above-mentioned effects could be the result of mere unspecific pollution in conditioned water. In this case we would also expect a delay of growth and differentiation in the offspring when raised in conditioned medium. However, 8 nauplii of the F1F2 females from Test 3 (above), raised in conditioned medium, reached metamorphosis and adult age at the same time as 8 control siblings in fresh sea water (mean of 7.7 days to metamorphosis and 20.3 days to adult in conditioned medium; controls 7.5 days and 19.9 days, respectively).

Tests from 1975/76: The A, B, C-series

Effect of conditioned medium on fertility (Table 3). Test animals of the three cultures (p. 211) were transferred into conditioned medium and the same number of controls were observed in fresh medium. From the C-culture two categories of harpacticids were assessed: (1) first breeders which came directly from a one-month-old, very high density culture, which, as far as may be told (p. 210), was nevertheless well fed; (2) individuals raised under low-density conditions as usual for test animals. This second category is directly comparable with the three tests from 1971 (Table 2, Fig. 1); the same conclusions apply to all groups in the A, B, and C-series: conditioned medium significantly reduces the number of ovisacs produced.

I had noticed on several occasions that in the B-culture and in any high-density culture an unusual number of eggs did not hatch, apparently their proteins coagulated, and they turned into a milky, opaque white, whereas fertile eggs are silvery and almost

Table 3. *Amphiascoides* sp. Reproduction in fresh and conditioned medium, 1976

Type of test individual*	Medium	Tests of 10 pairs	Number of ovisacs produced per 10 ♀♀ in 40 days	% hatched	Fertile periods (days of adult age)	F_1 (total produced per 10 ♀♀)
C_9^{hd}	Fresh	3	75	75.6	35–40	148
			$P < 0.01$	$P < 0.0025$		
C_9^{hd}	Conditioned	3	54	49.0	30–35	34
C_9^{ld}	Fresh	1	128	93.2	50–55	615
			$P < 0.01$	$P < 0.025$		
C_9^{ld}	Conditioned	1	90	75.9	50–55	324
A_9	Fresh	2	137	84.3	40–45	571
			$P < 0.01$	$P < 0.025$		
A_9	Conditioned	2	111	72.3	50–55	350
B_9	Fresh	2	98	70.0	30–35	410
			$P < 0.01$	$P < 0.01$		
B_9	Conditioned	2	67	54.5	25–30	175

* The indices in Tables 3 and 4 indicate the number of months the specific culture regime had lasted (p. 211)

transparent. Table 3 shows that sterilization of part of the eggs is a regular consequence of conditioned medium, the percent of eggs from which nauplii hatched is significantly reduced. The two effects, reduction of eggs and partial sterilization of the eggs actually produced, result in a drastic cut-down of the F_1 from females which breed in conditioned medium. These may be regarded as 'immediate effects' because females raised in fresh water at low densities also show them when transferred into high-density conditioned medium (C^{ld} and A). C^{hd} and B-females were raised in conditioned medium and set into fresh water to breed. They produced fewer eggs and a higher percent of sterile eggs than controls raised in fresh medium ($P < 0.01$ and < 0.025, respectively). Referring to a single generation period, these are long-term, irreversible effects.

B-females, although raised in conditioned medium, were nevertheless kept at low numbers during growth; in contrast, C^{hd}-individuals were exposed to high density, with large numbers of animals jostling for the same food supply, as well as to conditioned medium. This may explain the fact that C^{hd}-females produced considerably fewer ovisacs than B-females, both, in fresh and conditioned medium ($p < 0.05$). Furthermore, counts of the number of eggs per ovisac show that C^{hD}-females also produce smaller sacs: these contain on the average 11.3 eggs (74 ovisacs counted) as compared with 13.1 in B-females (45 sacs counted of females raised in conditioned water; t-test, $P < 0.001$). The ovisacs of A-females contain 14.1 eggs (all these numbers refer to single sacs, not to the pair). Reduction of eggs per ovisac is thus largely due to the density of animals on the same food supply and not to conditioned medium.

Lastly, growth and differentiation of *Amphiascoides* sp. in conditioned medium and at high density seems to reduce their fertile period by about 40 % (C^{hd} and B-females as compared with C^{ld} and A-females). The tests were not designed to determine the fertile period, these data are a mere by-product of the daily counts of ovisacs, hence, they do not lend themselves to statistical evaluation. There is one remarkable observation, though, with regard to breeding periods: if we compare these data with test No 3 from September 1971 (Table 2), we find that the F1F2 females bred over a period of 81 days. This is a further indication that the laboratory stocks changed their life cycle in the course of years (p. 212).

The combined effects of conditioned medium and high density – which are: reduction of number of ovisacs, reduction of fertile eggs per ovisac, extension of interval between release of successive pairs of ovisacs and reduction of fertile period – result in a total offspring of 3.4 per single C^{hd}-mother. Considering that the sex ratio is 1 : 1, and that the reduced fertile period allows for little overlap of generations, this brings fitness to the vicinity of 1. These data explain the repeated, but quite casual, observations that an aged, dense population cannot be substantially increased by abundant feeding and by the occasional, partial water changes which are necessary to remove excessive waste products.

Effect of conditioned medium on the release of ovisacs. Delayed release of ovisacs in conditioned medium, as shown by the three tests in 1971 was also verified in the A, B, and C-cultures. Young, breeding females carrying ovisacs were transferred directly from low-density cultures into observation dishes with conditioned and fresh water, respectively. During three subsequent days the number (not pairs) of ovisacs were noted. Table 4 gives the data per 50 females or, as each female initially carried one pair of ovisacs, in percent of ovisacs. C-females release their sacs more readily than either A ($P < 0.0025$, X^2-test) or B ($P < 0.001$);more sacs are released on the first day after transfer. A-individuals in fresh medium and B-individuals in conditioned medium represent the normal breeding conditions of these stocks; release of ovisacs is certainly delayed in the B-culture ($P < 0.0025$). A-females, when brought into conditioned medium, behave like B-females; however, B-females in fresh medium are even more delayed than when in their accustomed (conditioned) medium: 21 % less

Table 4. *Amphiascoides* sp. Release of ovisacs in fresh and conditioned medium

Type	Experimental females: Number of ♀♀ per number of tests	Medium	Ovisacs released per 50 ♀♀: 1st day	2nd day	3rd day	Total released
$C_{2,3,4}$	60/3	Fresh	57	22	15	94
$A_{2,3,4}$	120/6	Fresh	40	35	13	88
$A_{2,3}$	40/2	Conditioned	16	40	29	85
$B_{2,3}$	40/2	Fresh	23	30	16	69
$B_{2,3,4}$	90/6	Conditioned	22	48	20	90

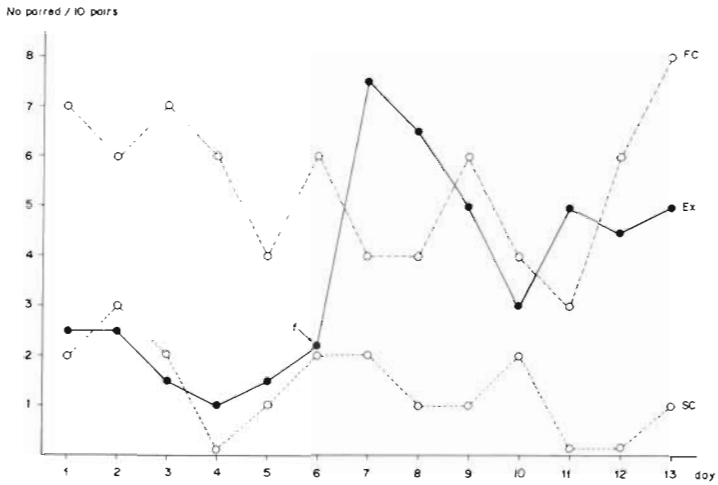

Fig. 2. *Amphiascoides* sp. Dynamics of daily pairing incidence as a function of food supply over an observation period of 13 d. FC: food control, one group of 10 pairs fed during whole observation period; SC: starvation control, one group of 10 pairs starved during whole observation period; EX: experimentals, means of 2 groups of 10 pairs each, fed only on the 6th d (f) and thereafter. Difference between any 2 curves: $P < 0.01$ (sequence test)

ovisacs are released within the three days. This shows again the long-term effect of conditioned medium on reproduction which cannot be reversed by bringing the females back into fresh medium. As nauplii hatch upon release of the ovisacs, and as new sacs can only be formed after release of the former pair, delayed release of ovisacs means delayed development with a respective increase of the generation period and decrease of fitness.

Effect of Food Supply on the Release of Ovisacs and on the Incidence of Mating

Effect of food supply on the release of ovisacs

Young, experimental females carrying ovisacs were isolated in groups of 10 on day zero and then starved for a definite number of days; the number of ovisacs released was noted every day. After this period the females were fed and observed for an equal period after feeding; again, the number of released ovisacs was noted. All harpacticids were kept in fresh medium. Controls were fed during both periods. The results are shown in Table 5. It may be stated almost categorically that ovisacs are not released in the absence of food; 85–100 % of the females detach their eggs only when they chance upon a food source. Females which are starved for 5 and more days seem to resorb part of their eggs, ovisacs become smaller and in cases disappear altogether. Another explanation may be that eggs rupture and that their content disperses into the medium, or that females begin to eat their eggs as reported by Marshall and Orr (1964) for *Calanus*; however, I never noticed anything to this effect. The controls produce their second and third pairs of sacs during the observation period whereas the experimental females are unable to recover their reproductive potential in the prolonged post-feeding period of 4–7 d. That a fresh food supply actually t r i g g e r s the release of ovisacs is particularly evident in the case of single-day deprivation of food, none of the females released its sacs during this day but most did on the second upon feeding, whilst the controls released them on the first day. Futhermore, 75 % of the females, which were starved for 4 d released their ovisacs within 24 h after food was provided again (not shown in Table 5).

Table 5. *Amphiascoides* sp. Release of ovisacs as a function of food supply

Observed periods (days)		Number of ♀♀ observed		Experimentals: ovisacs released per 10 ♀♀			Controls: ovisacs released per 10 ♀♀		
before feeding	after feeding	Experimentals:	Controls	before feeding	after feeding	Total	before feeding	after feeding	Total
1	1	40	40	0	11.50	11.50	9.75	3.25	13.00
4	4	30	60	2.67	19.67	22.34	18.00	10.17	28.17
5	5	20	10	3.00	11.50	14.50	14.00	25.00	39.00
7	7	10	10	3.00	14.00	17.00	28.00	22.00	50.00

Effect of food supply on the incidence of mating

In any mass culture of *Amphiascoides* sp. a fraction of individuals finds itself in pairs, the males being attached by their antennae to the tail furca of the females. Addition of new food to a culture nearing the exhaustion of its previous food supply results in a sudden rise of the number of pairs; this, at least, is the impression one gets during routine maintenance of the cultures. To test the hypothesis that meeting with a food source triggers pairing behaviour, small groups of experimental animals were observed: during an initial period they were deprived of food; on the sixth day they were fed and kept well fed thereafter. Two groups served as control: one that was fed throughout (food control) and one that was starved throughout (starvation control). At 24 h intervals the number of pairs present was counted. The product between number of pairs observed (coupled + uncoupled condition) and number of observation days represents 100 % of observation units. The sum of coupled pairs observed over all observation intervals (days) expressed as fraction of these 100 % of observation units is defined as 'pairing incidence'. It may be interpreted as the % pairs present in paired condition on a single day, or as the % days a single pair was found coupled over a given observation period. Figure 2 leaves no doubt that discovery of a food source after a period of deprivation acts indeed as a pairing triggers. It is important, though, that even in a population in starving condition pairing incidence fluctuates around 10 %.

Mating and Fertility

Group effect on the incidence of pair formation

In order to determine the dynamic pattern of pair formation in more detail, single young pairs of copepods were isolated into 10-ml Petri dishes with fresh medium and food. However, it was soon apparent, that these conditions did not match the high pairing incidence in mass cultures; isolated pairs remained in general separated. This was not due to a reduced probability of encounters between the sexes, because male and female spent most of their time on the small piece of lettuce leaf and hence, well within each other's sphere of perception. To detect a possible group effect on pairing incidence, single pairs, which had been observed for an initial period were subsequently grouped in 3–5 pairs and observed for a second period. Pairs kept in groups during both periods served as controls. The data of all similar tests are summarized in Table 6. Pairing is 5–7 times more intense in groups than in single pairs. Still, pairing incidence is 5 % in single pairs and thus presumably ample to guarantee insemination.

Pairing and fertility

To understand pairing behaviour as a function of food supply and social structure we must know whether fertility is dependent on repeated mating. As Figure 3 shows, this is decidedly the case. The drop of fertility at the adult age of 15–25 d also occurs if females are provided with males during this period. This is evident from a series of observations not presented in further detail: in two groups of 20 and 10 pairs with a pairing incidence of 40–60 %, ovisac production was reduced to 1/3 at this age and it ascended again into a minor peak later. Figure 3 shows that in females deprived of males fertility drops to zero and stays there. Yet, if supplied with males after prolonged periods of deprivation, already aged females produce belated peaks of fertility which partly compensate for the loss of reproduction in the earlier phase.

Reproductive Period and Longevity

The suspicion that reproductive losses due to limited deprivation of food and males might be compensated for by an extended fertile period appears justified if all data on fertile periods and longevity that became

Table 6. *Amphiascoides* sp. Group effect on pairing incidence

Condition	Experimental pairs: Number observed	Total observation units (pairs × days): 100 %	Pairing incidence (%)
Single pairs during first observation period	32	263	5.3
In groups of 3–5 pairs during second observation period	Same pairs as above	217	26.7
Controls: In groups of 5 pairs during 1st and 2nd period	20	145	36.6

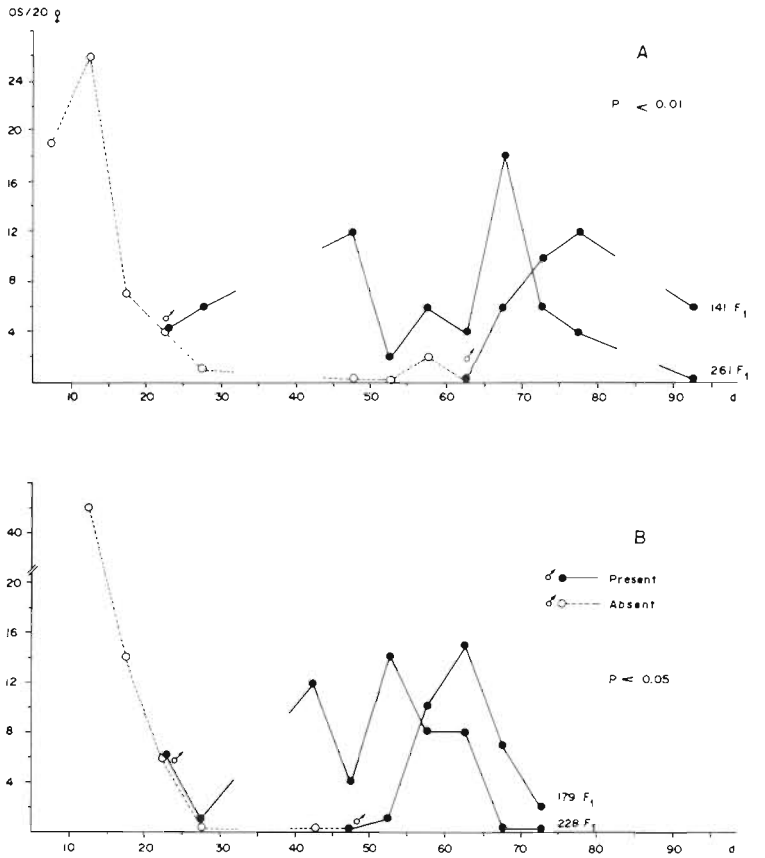

Fig. 3. *Amphiascoides* sp. Fertilization and fertility. Two series (A, B), each with 2 groups of 20 initially fertilized females. Males added later after various male-less periods. OS/20 ♂: total number of ovisacs produced by 20 females within successive 5-d periods. d: adult age in days of the females. F_1 = total offspring produced after age of 20 d. P = significance for difference between the 2 groups (sequence test)

available in the course of time are compiled in a table (Table 7). None of the observation series had been designed to determine the fertile period, hence the data refer really only to the one, longest producing female within the respective group. Still, it is evident that under laboratory conditions at least, the fertile period is much shorter than the mean longevity of approximately 4 months. Females provided with males and food throughout seem to spend half of their life span in sterile old age, whereas females deprived for limited periods seem to prolong their fertile period. The simplest explanation would be that females have a limited supply of oocytes and use these according to availability of food and mates.

As to deprivation of food a few explanations are in place here: in absence of fresh food, *Amphiascoides* sp. feeds on its own faeces. This became evident in females which had been fed with yeast dyed with methyl blue to mark them individually for a limited period (their intestine becoming blue). Such females were put together singly with a group of starved, undyed individuals which, in turn, acquired blue intestines and produced blue faeces. Deprivation of food does thus not mean absolute starvation, at least for some time. Still, in females starved for more than 15 d the ovaries become transparent as all protein disappears, the harpacticids turn more and more transparent and the normally vigorous movements slacken off.

DISCUSSION

Table 8 summarizes some of the more recent biological data on various copepod species bred in the laboratory (for review see 'Marine Ecology' volume III: Kinne, 1977). The data of Gaudy and Guérin (1977) include the various *Tisbe* species of Battaglia's laboratory*. We find that the sex ratio fluctuates around 1 : 1 in harpacticoids and calanoids alike, which points to a common genetic mechanism of sex determination. A

* Battaglia (1957); Battaglia (1970); Parise and Lazzaretto (1966); Volkmann-Rocco and Fava (1969); Volkmann-Rocco and Battaglia (1972).

deviation in favour of males at high population densities, as Heinle (1970) reports for *Acartia tonsa*, was never observed in *Amphiascoides* sp. Breeding difficulties arose sporadically in the A- and B-cultures, which were kept perpetually at relatively low densities and thus were subject to intense inbreeding. In such abnormal cultures pairing incidence and females with ovisacs became infrequent or ceased altogether However, the sex ratio was not determined. Still, the observation would suggest that *Amphiascoides* sp., a harpacticoid as *Tisbe*, suffers modification of the sex ratio in favour of males as a result of inbreeding, as Battaglia (1964) has reported for *Tisbe*.

Taking the various breeding temperatures into

Table 7. *Amphiascoides* sp. Reproductive period and longevity

Year	Experimental animals number	adult age (d)	Deprived of males (n° days)	food (n° days)	Last offspring within group after n° days	Longevity dead at adult age (d) 50 %	100 %
1975	10 pairs	12–18	0	0	56	132	160
1975	6 pairs	2–7	0	0	50	–	–
1975	5 pairs	2–7	0	0	62	–	–
1975	30 ♀♀ (fert)	2–7	Throughout test period	0	19	–	–
1975	30 ♀♀ (fert)	10–20		0	40	–	–
1971	7 ♀♀ (fert)	2–4		0	31	–	–
1975	20 ♀♀ (fert)	10–20	28	0	76	–	–
1975	20 ♀♀ (fert)	10–20	15	0	71	–	–
1971	5 ♀♀ (fert)	2–4	18	0	84	121	144
1971	6 ♀♀ (fert)	2–4	18	0	71	–	–
1975	8 pairs	?	0	28	74	107	133
1975	6 pairs	2–7	0	50	75	62	153
1975	10 pairs	12–18	0	65	111 (sterile eggs)	80	160

Table 8. Comparison of biological data from various copepod species bred in the laboratory. Numbers in brackets: data obtained by inference, not by direct tests

Species and authors	Longevity (inclusive development)	Duration of development	Fertile period	Pairs of ovisacs per female	Eggs per pair of ovisacs	Eggs per female	F_1 per female	Sex ratio (% females)
Tisbe holothuriae Gaudy and Guérin (1977)	23.6–33.4 d 19 °C	12 d	6.5–10.5 d	3.7–5.1			188–310	40–58
8 *Tisbe* species Gaudy and Guérin (1977)	23.6–81.4 d 18°–19 °C	12 d	10.5–23 d	5–9	31–78		128–513	33–60
Euterpina acutifrons Zurlini et al. (1978)		10–12 d 18 °C	19–40 d	12.5	16–26		295	39.8
Amphiascoides sp. (present paper)	131 d until 50 % dead 170 d maximum 23 °C	9–19 d	50 d (80–100 maximum)	10.5	23–28 (young females)	(150–300)	(61–100)	(50)
Acartia tonsa Parrish and Wilson (1978)	exclusive development: 26–43 d 18 °C		39 d maximum			450–1596		
Rhincalanus nasutus *Calanus helgolandicus* Mullin and Brooks (1970)		23 d 15 °C						~ 50
Pseudocalanus Corkett and McLaren (1978)	> 100 d 6 °–14 °C	46–63 d	5–43 d	3–16	25			~ 50

account, development periods show little differences. Battaglia (1970) reports 10 moults from egg to adult in *Tisbe*. This coincides with *Amphiascoides sp.*; however, *Tisbe* has 5 nauplius and 5 copepodite stages, whereas *Amphiascoides* has 4 and 6, respectively.

Values of reproduction are of the same order of magnitude within the harpacticoids, and are relatively low as compared with the pelagic *Acartia tonsa*.

However, *Amphiascoides* differs radically from other harpacticoids in longevity and in fertile period. As roughly 50 % of ovisacs are produced during the first month of its longevity, adult *Amphiascoides* survive 4–5 of their own generations. Theoretically, a female would still be alive, when some 50 000–100 000 of its female descendents populate the area. It can afford to lose most of its F_1 and F_2 and still produce a compensatory F_1. More important still, the enormous life span, the capacity to survive prolonged starvation and the flexibility of the reproductive period allow for regulation of reproduction in response to population density and availability of food and males. The data for *Pseudocalanus* (Corkett and McLaren, 1978) might suggest a similar pattern. Thus, they found that starved females lived up to 71 d. However, these authors worked with very low temperatures as compared with the culture temperature of *Amphiascoides* sp., and the data are therefore not comparable on a physiological basis. Ecologically, though, the two species might command similar strategies, each within its own, natural temperature range.

A proper regulation mechanism must correct both, positive and negative deviations from a programmed output (= sollwert); overpopulation and underpopulation are to be avoided. Thereby, the sollwert is not a fixed value, but has different optima depending on the amount of food per area. Such regulative mechanisms can only be evaluated in relation to the biology of the species.

As *Amphiascoides* sp. is new to research, the possible relationships between its biology and ecology, outlined in what follows, are partly hypothetical.

It is certain that firstly, *Amphiascoides* sp. lives in the tidal zone with its sand flats, coral reefs and tidal pools, where food sources are patchy and where accidental dispersal by waves and currents must be frequent; secondly, *Amphiascoides* sp. is essentially a plant-detritus feeder; and thirdly both sexes are freely mobile, and continued breeding needs repeated insemination. Consequently, the harpacticid may have to solve the following problems: to survive prolonged, foodless periods of passive disperal; to implant a colon-izing group in a food patch accidentally met with; to secure mates and to avoid over-exploitation of isolated habitats.

In free living organisms the highest possible frequency of chance encounters between males and females is 50 % at a sex ratio of 1 : 1. Continuous inbreeding in dense and stable groups would allow for devious sex ratios to develop, for example a reduction of males, while outbreeding opposes such deviation, firstly because a species which preserves the 1 : 1 ratio presumably has a higher breeding success, and secondly because recombination of genotypes is more intense within a mobile and more dispersed group; accidental aberrations would have little chance to accumulate, in other words, selection, as a result of relatedness, would be low (Hamilton, 1972). In *Amphiascoides* sp. pairing incidence is higher if harpacticids are in groups than if they are isolated in pairs, and patchy food distribution favours ephemeral group formation. This probably results in multiple insemination of a single female by various males, and thus in maximum genetic variation among the offspring of a single female. The tenacious pairing behaviour, in that males remain attached to females for many hours, ensures that many adults disperse in pairs. Longevity and the capacity to survive periods of starvation, together with the possibility of deferring fertile periods, favour the chances of dispersed individuals or pairs colonizing habitats accidentally encountered. The most efficient mechanism to this end is the formation and release of ovisacs. These are carried along and are ready to be released when a new egg supply is mature to form the next pair of sacs. Thus, during a fertile period, females have a reserve of mature eggs almost permanently, despite the fact that eggs can only be laid in discrete batches; the release is triggered by the finding of a food source. Hence, while the coincidence of harpacticid and food may be left to chance, the placement of the eggs is strictly coordinated with food availability; no eggs are wasted in unsuitable places.

This mode of oogenesis and embryogenesis is more complex than meets the eye insofar as hatching of the nauplii is correlated with the release of ovisacs. It is, in fact, almost a case of ovoviparity with all its maternal protection for the developing embryo. There is, in addition, the relation between number of eggs per ovisac and shortest interval between release of subsequent pairs of ovisacs. The opportunist would be expected to form smaller sacs at shorter intervals. In this respect it is interesting that the C-stock, which is oscillated through low and extreme densities, produced smaller, but more readily detachable ovisacs than the Stocks A and B, which were kept in more stable conditions.

All these mechanisms compensate underpopulation; they promote speedy colonization and enable a disturbed population to accelerate recovery.

Some of the very same mechanisms reduce popula-

tion increase at high densities, such as retention of ovisacs in absence of food and in medium conditioned by high population density, together with delayed hatching of the F_1. In addition, there is the abbreviation of the reproductive period in females subject to high density with partial sterilization of their eggs. Combined with the non-lethal, but nevertheless delayed density-dependent effects (reduction of reproduction in the F_1 that developed under conditions of high density) *Amphiascoides* sp. approaches stability ($r \simeq O$) at conditions of extreme density; this occurs even in the absence of predation and accidential loss, both of which are undoubtedly high in nature. Thus, the reducing regulators seem to be more than adequate to prevent collapse of a population due to over-exploitation of the habitat. As long periods of isolation in closed water bodies are exceptional in the tidal zone, conditioning of the medium (supposedly by pheromones) may rather have the function of inducing active dispersal. Conditioning of the medium, intensified pairing when in groups and attachment of males to females for extended periods would then constitute an integrated mechanism of dispersal and crossbreeding as a function of local density.

In order to estimate the power of regulative mechanisms quantitatively, series of life tables at various densities in relation to food and water volume would be necessary, as well as the establishment of a dispersal function in relation to density. The range of *r*-values thus established would represent the capacity of phenotypic stress absorption by a species or population. The ideal population density would lie somewhere in the middle of tolerable limits where enhancing and reducing population regulation mechanisms function free of friction, causing the minimum of delayed effects. Such an approach would lead to a more realistic assessment of population dynamics than the tradition of calculating future population densities as a function of minor ripples in the infrastructure of reproductive chance and behaviour. Population models with constant reproductive rates are clearly of exclusive theoretical interest. With regard to the evolutionary prospects the situation is reversed: individual ripples of *r*-values in different genotypes determine the quality of genes which will be selected, whereas the regulated population stabilizes the level of competition and thus of the selection pressure, and thus prevents non-linear, qualitative switches in the genetic selection system (switches to aggression and defense or to mere drift as a result of population collapse for example).

One further point with regard to evolutionary prospects needs to be mentioned. Regulated reproduction and dispersal are the result of dynamic processes: more or fewer eggs are produced and dispatched earlier or later; development is more or less delayed; emigration is more or less intense. It had been shown in previous studies (Walker and Williams, 1976; Walker, 1979) that environmentally induced dynamic patterns will inevitably be fixed by irreversible accumulation of genetic effects if there is no active selection for their flexibility. Thus, the extreme flexibility of *Amphiascoides'* reproductive, density-dependent behaviour indicates its selective value. Furthermore, the A and B-stocks, kept at stable low and high densities, respectively, showed reasonably stable, specific reproductive patterns. These regimes lasted for 9 months only, and genetic loss of flexibility can hardly be expected in this short period. However, the different patterns indicate the long-term direction of a genetic change, if the same conditions were to continue. It is possible, furthermore, that the almost 10 years of laboratory breeding of the laboratory stock under comparatively high densities (as compared to natural conditions) led to the marked change of the life cycle (Table 1); in other words, extended developmental period (mathematically far more powerful than reduction of birth rate, Malthus, 1803; Rabinovich, 1968) and sterility during the later part of life may be laboratory artefacts in the process of genetic fixation. In this respect it is interesting that Corkett and McLaren (1978) obtained fertile ovisacs for 80 d from *Pseudocalanus* females collected in the sea, yet females raised in the laboratory had a maximum fertile period of only 43 d and remained sterile for up to 70 d.

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