

Reproductive ecology of the saltmarsh-dwelling marine ectoparasite *Paragnathia formica* (Crustacea: Isopoda)

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Adults of the isopod *Paragnathia formica* inhabit burrows in saltmarsh banks from which they release larvae during autumn high tides. Larvae pass through three moult stages, each of which feeds ectoparasitically on estuarine fish (including *Pomatoschistus microps*), before a final moult to a non-feeding adult stage. The entire energetic reserves for survival and reproduction up to nine months (females) or 16 months (males) later are therefore acquired during these three brief periods of parasitism. Application of a plankton sampling technique showed larval density in the water to vary considerably between successive high tides. High densities of larvae (1 per 1.2 l) occurred on only one tide during a week-long study, when a corresponding peak in parasite prevalence in the fish population (10%) was recorded. Peak larval release was observed as the tide rose, at a time when host fish have been reported to be in greatest abundance. Considerable larval longevity was demonstrated in the laboratory in the absence of food (mean=43 d). Data are interpreted, in conjunction with field observations, in relation to larval parasitism opportunities post-birth.

INTRODUCTION

The saltmarsh-dwelling isopod *Paragnathia formica* Hesse, a member of the Gnathiidae, is both viviparous and semelparous (Monod, 1926). The life cycle comprises three free-swimming haematophagous larval stages and a non-feeding reproductive adult. Life-time nutritional intake is limited to three brief periods when larvae are ectoparasitic on estuarine fish (Upton, 1984). These three meals must provide fully for adult development and homeostasis during a maturation period of up to nine months in the case of females, thus placing tight constraints on the energetic reserves available for reproduction. High pre-birth investment, involving complete development *in utero*, enables larvae to exploit infection opportunities immediately following parturition and dispersal. Poorly mobile larvae have to attach to comparatively fast swimming hosts in a relatively vast expanse of estuarine water. Therefore the larval release strategy and progeny resource partitioning adopted by a parent must maximize fitness in spite of highly uncertain individual offspring fate.

Detailed observations on the life cycle and developmental stages of *P. formica* have been made (Monod, 1926; Stoll, 1962; Upton, 1984, 1987a). Males dig burrows in saltmarsh banks to which they recruit harems of 1–25 females during early summer (Upton, 1984). Burrow entrances normally exclude water during submergence (Upton, 1984). However, in autumn, when females are gravid, males excavate openings at high tide flooding the harem. Contact with water initiates parturition when the female body wall ruptures, releasing larvae (Upton, 1987a). These parasitize estuarine fish, such as the common goby (*Pomatoschistus microps* Krøyer), the infection period ranging from two to 24 h (Stoll, 1962). Fed larvae return to saltmarsh banks and make small burrows in which to moult. The pre-feeding and post-feeding forms are morphologically distinct and are termed zupheas and

pranizas respectively (Stoll, 1962). Pranizas moult, over a three-week to three-month period, to second stage zupheas which undergo two further rounds of parasitism. Upton (1987a) described these forms using the abbreviated terms Z1, P1, Z2, P2, Z3, P3, to denote the moult phase and feeding status of individuals.

Early settling third stage pranizas (late autumn to late spring) generally become females, and are recruited to the burrows of males from the previous generation. Pranizas settling later (early summer onwards) become males and either excavate new burrows or occupy vacant ones. Larval settlement is gregarious, males being attracted to burrows already containing adults (Upton, 1987b). Whilst females breed and die one year after birth, males wait and survive a second winter to recruit harems the following summer. Despite this clear trend in settlement timing little is known of the sex determination mechanisms in *P. formica* and how this asynchronous settlement pattern is driven (Upton, 1987a).

Paragnathia formica larvae will parasitize a variety of hosts, however the common goby (*P. microps*) is frequently employed (Upton, 1984). This species occurs in high densities in shallow estuarine waters (Fish & Fish, 1996) and feeds benthically (Antholz et al., 1991), with crustaceans forming a major dietary component (Healy, 1972). Thus gobies are both a host and predator of *P. formica*, creating an unusual challenge to larvae attempting infection.

Previous studies of *P. formica* have focused on its remarkable morphology (Monod, 1926), life cycle and harem formation (Upton, 1984, 1987a,b). Upton (1984) monitored infection levels in the fish population to infer larval release trends. However, no attempts have been made to quantify larval release directly.

This study aims to document several key aspects of *P. formica* reproductive ecology. It is here demonstrated that larvae can be recovered from, and their density quantified in estuarine water, by plankton net filtration. These

observations and data on host fish infection levels are presented with the aim of determining release tactics. Larval longevity is also interpreted in relation to host infection opportunity.

MATERIALS AND METHODS

Study site

Fieldwork was carried out between 31 August and 8 September 1998, on a small saltmarsh 750 m east of the harbour at Wells-next-the-sea, north Norfolk, UK (OS grid reference TF 925438). This site is on the south side of the main drainage channel from the Wells and Warham saltmarshes. A 1.5 m high saltmarsh cliff rises from a band of waterlogged mud forming the boundary of the main channel, a 100 m wide stretch of sand. Pools form in the channel at low tide containing common goby shoals. Maximum tide height was measured daily relative to the top of the bank, using a calibrated cane. Comparisons with tide tables for Immingham enabled the bank top to be estimated at 3.3 m above chart datum. High tide height increased approximately 40 cm d⁻¹ throughout the study, during the change from neap to spring tides (Table 1). New areas of bank were submerged during each high tide until 5 September when tides rose above the top of the bank.

Larval density variation during successive tides

Abundance of *Paragnathia formica* larvae in the water was measured during consecutive tide cycles, to detect larval release from harem burrows in the creek cliff. Water samples, each of 640 l (75 buckets) were taken from surface waters within 1 m of the bank and filtered through a 200 µm plankton net. Five high and two low tide samples were taken, each on successive days during the study period. Filter residues were washed from the net; large particles were removed using a coarse filter (mesh size 4-mm) and excess water by density separation. Sub-samples

Table 1. Variation in larval density in estuarine water and parasite prevalence in the fish population. Parameters were estimated by plankton net filtration of 640 l of water and samples of 100 fish respectively, on the days indicated. No comparable sample was taken on 5 September 1998 when the density variation during a tide cycle was investigated (Figure 1). Tide height was measured relative to the bank top, + indicates that tides rose above the top of the bank, flooding the saltmarsh. Prevalence and density both peaked simultaneously on 4 September 1998.

Date	Tide status during sampling	Maximum daily tide height (cm)	Number of larvae in 640 l	Prevalence (%)
31/08/1998	Low			1
02/09/1998	High	-124	41	1
03/09/1998	High	-85	1	-
04/09/1998	Low		15	-
04/09/1998	High	-41	516	10
05/09/1998	High	+9	-	-
06/09/1998	Low	+	97	0
07/09/1998	High	+	6	3
08/09/1998	High	+	7	3

were decanted into Petri dishes and *P. formica* larvae identified and counted under a binocular microscope (×12 magnification). Samples taken after 4 September were preserved in 70% alcohol and analysed later. Larvae were not generally classified further; however, of 110 larvae inspected (collected 6–8 September) all were Z1 zupheas except for one praniza.

Parasite infection levels in the common goby population

Gobies were sampled from shallow waters (N=100) using a pond net, approximately every two days during the study period. Samples were taken as the tide fell, thus representing fish exposed to larvae released during the most recent high tide. Within 3 h of capture, fish were screened for *P. formica* infection, using a binocular microscope (×6 magnification). Parasite burdens and attachment sites were recorded.

Variation in larval density during a low–high tide cycle

Larval density in the water was estimated over the course of a single tide cycle. Sampling methods were identical to those above, except that 425 l (50 bucket) samples were taken. This enabled faster sampling, increasing sensitivity of measurements to temporal changes in larval numbers. Eight consecutive samples were taken from the surface water adjacent to the channel bank. Tide height and time were recorded before and after each sampling period. Larvae recovered were prepared as above and preserved in 70% alcohol. All larvae were classified as zupheas or pranizas (as adopted by Stoll, 1962), and into their three moult phases, Z1, Z2, Z3 and P1, P2, P3 respectively (descriptions in Upton, 1987a).

Adult distribution in the channel bank

Seven mud samples (200 cm²×10 cm) were cut at 20 cm intervals along a vertical transect up the channel bank, to determine the distribution of *P. formica* adults. Samples were dissected and the number of male and female adults each contained recorded. Observations of water content and composition were made.

Larval longevity

Mud samples were collected from Wells on 1 October 1998 and stored at 4°C for five weeks before dissection to recover *P. formica* females. The broods of nine females that gave birth when placed in seawater were counted. A sample of seven larvae was taken from each of these broods and these larvae were placed individually into capped vials containing 10 ml of seawater. Larvae were maintained at 16°C without food and numbers surviving recorded every 2 d for the following 91 d. Inactive larvae were inspected under a binocular microscope (×25 magnification). Those not responding to water agitation, nor moving during a 5 min observation period were classified dead. Mean survival time was determined for each brood and analysis of variance used to investigate differences in longevity between broods. Larval lifespan was recorded conservatively as the last date observed alive.

RESULTS

Larval density variation during successive tides

Larval density was generally low, below 50 larvae per 640 l in five of seven samples during the study week (Table 1). However, 516 larvae were recovered from the sample taken during high tide on 4 September (tide height -41 cm), a density of one larva per 1.2 l. Low larval numbers (15 in 640 l) were recorded 5 h before this peak, in a low tide sample. The sample on 6 September (low tide) contained moderate numbers of larvae (97) and low densities were recorded on 7 and 8 September (6 and 7 larvae per 640 l respectively). Larvae were non-randomly distributed between high tide samples ($\chi^2_{v=4}=1776$; $P \ll 0.0001$).

Parasite infection levels in the common goby population

Gobies occurred in abundance in inter-tidal pools and were observed shoaling in shallow water at high tide. In five samples of 100 gobies, caught from 31 August to 8 September, parasite prevalence ranged from 0 to 10% (mean \pm SE = 3 ± 1.82 ; median = 1) (Table 1). Infected fish were non-randomly distributed between high tide samples ($\chi^2_{v=2}=9.57$; $P < 0.01$). Peak prevalence (10%) was recorded on 4 September; in all other samples prevalence was 3% or less.

Infection intensity was generally low; most hosts (22/26) carried a single larva. Three fish each carried two parasites and a single fish carried eight. Of the total parasite sample (N=36), 14 were attached to the fins and 22 to the body wall.

Variation in larval density during a low-high tide cycle

Sampling took place on 5 September, beginning at 1645 before the tide submerged the bottom of the channel bank. Samples were taken approximately every 20 min for 2 h as the tide rose, then a further two as the tide fell. Times represent the mean time for samples; each took approximately 10 min to filter 425 l.

The Z1 zuphea and praniza numbers changed considerably during the 3 h of sampling (Figure 1). Sample one (at 1650) contained moderate numbers of Z1 larvae (13). The Z1 larval numbers rose to a peak of 35 by 1750, as the tide rose between 35 and 20 cm below bank top (Figure 1). A gradual decrease in Z1 larval density occurred over the following 2 h, falling below ten larvae per 425 l. One Z2 larva was present in each of samples 3, 4, 5 and 8; these must have been born prior to 5 September. Density of P1 pranizas was initially high (33 per 425 l at 1650), then fell during the following 1.5 h to zero (Figure 1) and subsequently remained low (below 2 per 425 l). All pranizas sampled were P1 larvae apart from two P2 found in sample 3 (1730).

Adult distribution in the channel bank

On 5 September seven mud samples were taken spanning the full height of the channel bank. At this time the maximum high tide had reached 16 cm below bank top. All samples had a male-biased sex ratio, the extent of which varied along the transect; however, consistent patterns were absent (Table 2). Sex ratio bias was most extreme in samples between 20 and 70 cm (all above 0.7). This bias was less pronounced in the 0–10 cm sample

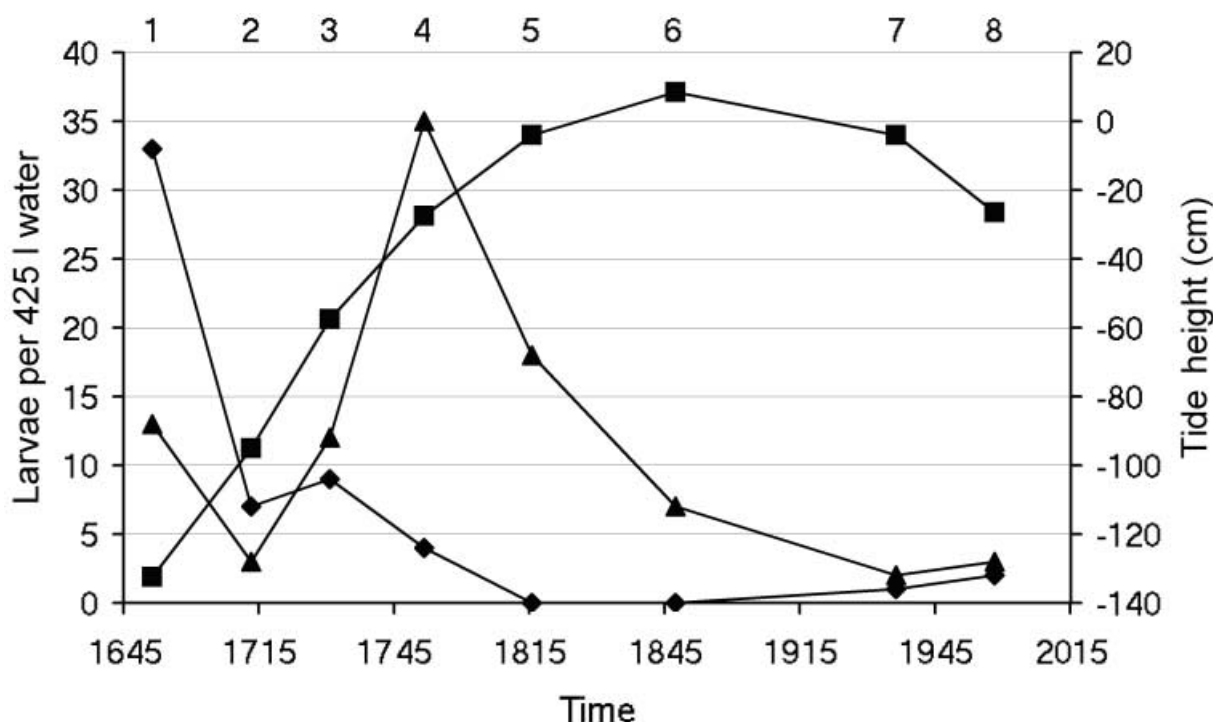


Figure 1. Changes in zuphea (pre-feeding) and praniza (post-feeding) larval numbers with tide height during a low-high tide cycle. Tide height shown by ■; number of Z1 zupheas per 425-l sample by ▲; and numbers of P1 larvae by ◆. Both tide height and time are means for the sampling period concerned. Praniza density was initially high, then fell. Zuphea density peaked whilst the tide was rising and fell as it rose further.

(0.64), and also samples 80–90 and 100–110 cm (0.65 and 0.55 respectively). Absolute numbers of adult *Paragnathia formica* showed no consistent trend above 110 cm, but decreased markedly in the 120–130 cm sample (Table 2). Overall distribution of adults between samples was non-random ($\chi^2_{v=6} = 51.4$; $P < 0.01$). Above 20 cm the sediment was relatively dry, containing much sand and organic matter. Between 20 and 110 cm, samples were of finer material increasing in water content lower down the transect. Below 110 cm, the mud was grey, waterlogged and anoxic.

Broods were counted for a sample of 37 females; mean brood size was 82.4 (SE ± 4.63 ; N=37) with a wide range (12–136) and considerable negative skew to the population frequency distribution (Figure 2).

Larval longevity

Survival time for neonate larvae ranged from 16 to 89 d, mean 43.1 d (SE ± 1.68 ; N=63). The survivorship curve for these larvae over time is sigmoidal (Figure 3) with the majority living for between 25 and 60 d. Mean survival period varied considerably between broods from 31.4 d (SE ± 2.21) to 59.9 d (SE ± 6.49). Significant inter-brood differences were demonstrated by analysis of variance ($F_{(8,54)} = 7.51$; $P < 0.00001$). In most brood groups (5/9) the interval between first and last death was less than 25 d; thus standard errors for sample means are relatively small

Table 2. Adult distribution in the channel bank. Clear trends are absent, however, absolute adult numbers appear to decrease markedly below 120 cm. Sex ratio was calculated as the proportion male. All sex ratios were male-biased, however this was most pronounced over the 20–70 cm height range.

Sample height (cm)	Number of males	Number of females	Total adults	Sex ratio
0–10	25	16	41	0.61
20–30	40	10	50	0.80
30–40	23	9	32	0.72
60–70	14	3	17	0.82
80–90	17	11	28	0.61
100–110	29	16	45	0.64
120–130	4	0	4	1.00

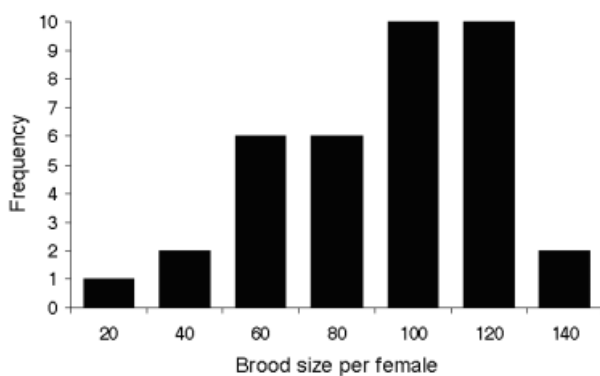


Figure 2. Frequency distribution of female brood sizes (N=37). Mean brood size 82.4 larvae, range 12–136. Considerable negative skew is apparent.

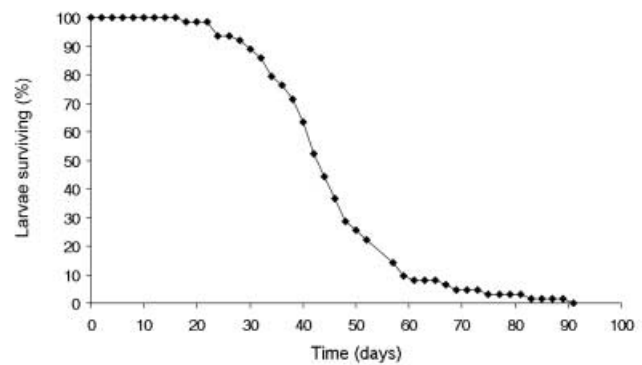


Figure 3. Survivorship curve of the larval population irrespective of brood group (N=63). Mean lifespan is 43.1 d (SE ± 1.68). The sigmoidal curve indicates an approximate normal distribution of larval survival times.

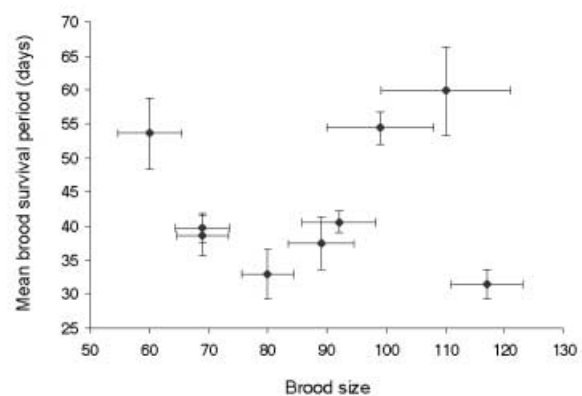


Figure 4. Relationship between brood size and mean brood survival period. A U-shaped distribution exists between the two variables, with one outlier at high brood size. Y error bars represent standard errors of survival period means, sample size for each brood was seven larvae. X error bars show scaled comparisons of one aspect of female fitness; total offspring infective opportunity (mean brood survival time \times brood size) in terms of total larvae hours. Data for broods from females of highest fitness occur at the top right: these individuals able to invest in both numbers and longevity of offspring. By this measure, the lowest fitness female has a brood size of 80 and achieves minimum brood survival.

(Figure 4). Larvae generally remained active until less than one week before death, after which only antennae and leg movements indicated life.

Brood sizes in this experiment ranged from 60 to 117 larvae. No linear correlation exists between brood size and mean longevity (Spearman rank correlation, $r_{s(n=9)} = -0.088$; $P > 0.1$), although there is an approximate U-shaped relationship (Figure 4). Mean longevity was high at both low and high brood size; intermediate brood sizes and the largest brood displayed low survival. Female fitness, with respect to total potential infection opportunities of offspring, was estimated by multiplying mean survival period by brood size (see Figure 4).

DISCUSSION

All of the first four larval stages of *Paragnathia formica* (Z1, P1, Z2 and P2) were recovered from seawater by filtration. No previous field records of free-swimming

larvae have been found in the published literature. The plankton sampling technique enabled quantitative analysis of the larvae present in the water at the site. Very low incidences of Z2 and P2 larvae were recorded (6 of 155 larvae classified), consistent with previous observations that they are not abundant in sediments until later in the year (Upton, 1987a). Complete absence of Z3 and P3 larvae reflects insufficient time since the release of the first Z1 larvae in mid-July for this moult stage to be present by early September (Upton, 1987a).

Variation in larval density during successive tides

That larval numbers varied significantly between daily samples strongly suggests that release of larvae from *P. formica* harems is influenced by tide height. Rising high tides during the study inundated new areas of bank each day not submerged during the previous week. Females at these sites, which had become gravid during this period, would have been ready to give birth. Upton (1984) has drawn similar conclusions from observations of *P. formica* prevalence in the fish population.

Larval density was relatively low during low high tides (Table 1). The density peak observed on 4 September may indicate massive larval release during that evening's high tide. This maximum decayed slowly over the next two to three days. This suggests either that larvae are not washed away and persist between tides, or that additional larvae released on subsequent high tides may offset those dispersed from the site. High zuphea density was observed in a low tide pool on 6 September, confirming that larvae do remain in the channel as the tide falls. Thus larvae may exploit further infection opportunities during future high tides and in inter-tidal pools where goby shoals occur.

Gobies are both hosts and predators of *P. formica* larvae. Upton (1984) proposed that simultaneous release of total harem progeny may be adaptive, increasing parasitism success and decreasing capture rates by both saturating (Pulliam & Caraco, 1984) and confusing (Neill & Cullen, 1974) predator populations. Sampled larval density on 4 September was one larva per 1.2 l. However, due to incomplete mixing and gregarious sibling dispersal (Upton, 1984), peak densities were probably much higher. Sufficiently high densities to confuse and saturate predators may therefore indeed occur in the field. Local release synchrony could result from clustering of adult burrows (observed by Upton, 1987b); then many burrows would be submerged during the same high tide. If this were the case, settlement of male P3 larvae in or near existing burrows (Upton, 1987b) would act to reinforce this effect each year.

Parasite prevalence and intensity

Peak prevalence coincided with peak zuphea density on 4 September and a causal relationship is proposed. Variation between neap and spring tides means that most *P. formica* burrows have periods of about a week between each series of submergence tides, probably causing pulses of larval release. It seems likely that the goby population suffers similar waves of parasitic infection throughout the *P. formica* breeding season.

Parasite prevalence was generally low (mean 3%), as were most observations of larval density. In addition the

majority of infected fish (85%) carried only a single larva. This may indicate that infections are predominately the result of chance encounters between larvae and fish and provides no evidence for variation in susceptibility. The single eight-parasite burden is higher than the maximum of four recorded by Upton (1984), but our prevalence estimates are similar. Ectoparasites may detach from hosts between capture and inspection (Grutter, 1995). Infection intensity and prevalence estimates here could therefore be conservative, but remain comparable.

Larval density variation during a low-high tide cycle

Density of Z1 larvae peaked and then decreased during the tidal cycle (Figure 1). Larvae in sample one must have been released during previous high tides as the water was below the level inhabited by *P. formica* adults (Table 2; and Upton, 1984). The immediate fall in Z1 larval density mirrors that of pranizas and probably results from dilution as the tide rose (Figure 1). Low zuphea density in sample 2 suggests that most larvae recovered subsequently were released during the tide cycle studied.

Channel bank sites not submerged during the eight days prior to fieldwork (above -20 cm), were predicted to contain many gravid females, ready to give birth on submergence. However, zuphea density peaked before the tide reached this level (-35 to -20 cm), reducing as the tide rose further (Figure 1). These data suggest burrows are not always opened on their first submergence and that further release from levels submerged during the previous day's exceptional density peak may have occurred. That males do not open burrows during their first submergence could be adaptive and ensure adequate immersion time for larval dispersal.

The zuphea density decrease as the tide progressed suggests that larval release slowed and that most larvae are released as the tide rises. This strategy may increase zuphea parasitism success. Healy (1972) has suggested that common gobies feed mainly on the rising tide, a behaviour documented in the sand goby (*Pomatoschistus minutus* Pallas) (Healy, 1971). Release as the tide rises and rapid onset of parturition on immersion of females in water (Upton, 1984) may be necessary to exploit host shoals which pass burrow entrances at this time.

Larvae attach to hosts for a minimum of 2 h (Stoll, 1962) before dropping off as pranizas post-feeding. Therefore, pranizas found in samples one to six originated from infections prior to this tide cycle. High praniza density in sample one may be the direct result of high prevalence (10%) and the larval density peak (516 in 640 l) during the previous evening's high tide. Tidal dilution, praniza settlement in the saltmarsh bank and negative phototaxis (Upton, 1984) could all account for the decrease in density as the cycle progressed.

Adult distribution in the channel bank

Previously submerged sites, from which larval release had occurred, were predicted to have a higher sex ratio (proportion male) because females are ejected from burrows after parturition. All sex ratios were male-biased, as reported by Upton (1987a) at this time of year (due to different male and female longevity and settlement timing). The sex ratio was

relatively low (0.61) in the sample taken above maximum tidal extent and as predicted was more pronounced in the three samples below this (Table 2). However, this trend does not continue lower down the transect where the bias decreases. Upton (1987b) has reported that harem size increases and male density decreases lower down saltmarsh banks. Therefore the trend observed could result from two confounding influences: larger harem size decreasing the male bias and female ejection increasing the bias at lower bank levels. A much more intensive sampling programme would be necessary to investigate this hypothesis fully.

The brood size–frequency distribution among the females sampled was clearly negatively skewed. It is likely either that a costly trade off exists against increasing brood size above 120 larvae, or that this represents a physiological constraint for most individuals.

Larval longevity

Larval death is assumed to have resulted from depletion of limited energy resources. The effects of toxic build up of excretion products or anoxia are unlikely because water volumes were relatively large and vials opened regularly during inspection. Whilst field energy budgets may be greater than those in the laboratory, it is evident that post birth survival is considerable (mean=43.2 d). It is likely that the potential infective period of zupheas is considerably shorter than total survival time, as has been demonstrated for infective stages in other systems (e.g. Anderson & Whitfield, 1975). Thus the field significance of this considerable longevity warrants further investigation. Linked peaks in zuphea density and parasite prevalence suggest that some larvae parasitize hosts rapidly on emergence. However, the extended survival period provides opportunities for infection at least days, possibly weeks, after parturition. The necessary resource allocation to larvae by females would be unlikely if this did not increase larval parasitism success. Indeed, larvae are provisioned adequately to adopt a 'sit-and-wait' strategy when host density is low and chance of active location remote. Relatively high numbers of Z1 larvae observed at low tide heights, originating from release during previous tidal submergences, may reflect this.

Significant inter-female variability in mean brood survival period was revealed. Whilst a genetic component to longevity may exist, the extent of reserve acquisition by females during their larval development must be a dominant determining factor. It is likely that female reproductive strategy involves trade-offs between larval provisioning and brood size. However, no clear correlation exists between brood size and mean brood survival period (Figure 4). It is possible that different reproductive strategies exist in females. Many poorly provisioned larvae would maximize reproductive fitness when host density and infection opportunities are high. Alternatively, fewer well-provisioned larvae could be advantageous to metabolically limited females if host encounter rate is low. Those females with greatest energetic reserves may be able to invest in both factors. Our data (Figure 4) suggest that both these strategies may occur.

The investigations presented here emphasize the highly unusual nature of the *P. formica* life history strategy. Previous authors have described its peculiar morphology, life cycle, settlement patterns and harem formation. We

have studied the reproductive phase in detail. Birth and subsequent dispersal of larvae have been quantified, demonstrating that high larval densities may occur in the sea, the result of massed release from many harems. Parasite longevity has been investigated, revealing a considerable window of infection opportunity. *Paragnathia formica* is the only member of the Gnathiidae in which true viviparous offspring development occurs completely *in utero* (Upton, 1987a). The resources for this development are derived from a brief parasitic phase up to nine months prior to reproduction (Upton, 1984). In this light, the extensive post birth survival period seems remarkable. The extent of nutrient gain during this parasitic period may strongly determine brood size, Z1 larval lifespan and vigour. These aspects of reproductive and larval ecology provide an insight into the significance of offspring investment decisions in determining parental fitness.

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