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EGG SIZE AND CLUTCH SIZE IN *PYRRHOSOMA NYMPHULA* (SULZER) (ZYGOPTERA: COENAGRIONIDAE)

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No significant relationships were found between either mean egg weight or clutch size and measures of female skeletal size, when controlling for the effects of sample date. However, for particularly large clutches, abdomen size may have had a limiting effect on clutch size. Mean egg weight and clutch size were both significantly negatively correlated with sample date. Body size declined through the season, but females that visited the study ponds late in the season laid smaller clutches of smaller eggs than those that arrived early in the season, irrespective of size. Possible explanations for this are discussed. In particular, climatological factors may exert a strong influence on egg production and deposition in damselflies and dragonflies. Clutch size is likely to be closely related to the inter-clutch interval, and therefore to the pattern of sunny and cloudy days during a female's reproductive lifespan, since reproductive activity occurs only on warm, sunny days.

INTRODUCTION

The majority of recent studies of short-term and lifetime mating success of damselflies and dragonflies have concentrated predominantly on males (FINCKE, 1982; BANKS & THOMPSON, 1985; HARVEY & CORBET, 1985; KOENIG & ALBANO, 1987; VAN BUSKIRK, 1987). Such studies have provided valuable insights into how factors such as size, date of emergence and longevity influence mating success. Lifetime studies, in particular, have emphasized the role of sexual selection in determining reproductive success. In addition, their advantage over short-term studies has been in indicating the importance both of opposing selective forces acting, for example, on longevity and survivorship, and extraneous factors such as chance and prevailing weather conditions (FINCKE, 1982; BANKS & THOMPSON, 1985; THOMPSON, 1990).

In contrast, studies of the reproductive activity of female odonates have tended to concentrate mainly on the costs and benefits associated with oviposition site selection (UBUKATA, 1984; BUSKIRK & SHERMAN, 1989), the characteristics and consequences of oviposition behaviour, and the adaptive significance of sperm competition and postcopulatory guarding (WAAGE, 1984, 1988; FINCKE, 1986), and oviposition duration and egg deposition rates (BICK et al., 1976; WAAGE, 1978).

Few studies of female damselflies have focussed on lifetime reproductive success to the same extent as those on males. In one such study of the damselfly *Coenagrion puella,* BANKS & THOMPSON (1987a) found that most variation (70%) in reproductive success arose from variation in survival rather than variation in fecundity. Females maximized their lifetime egg production by minimizing the interval between clutches, rather than by maximizing the size of each clutch. BANKS & THOMPSON (1987a) found that clutch size was inversely related to body size for one-day clutches, but that this relationship broke down for clutches with inter-clutch intervals greater than one day. In addition, they found no correlation between egg size and female skeletal size.

In this paper we present a study of factors affecting egg size and clutch size in another coenagrionid, *Pyrrhosoma nymphula.* This study complements that of GRIBBIN & THOMPSON (1991) who looked at male reproductive activity in *P. nymphula,* but falls short of the study of BANKS & THOMPSON (1987a) in not measuring lifetime reproductive success for reasons which are explained.

METHODS

The study was made in May and June 1988 at two ponds (areas 217 m^2 and 192 m^2) located in open mossland 40 m apart in Risley Moss Nature Reserve, Cheshire, northern England (N.G.R.SJ665915). *Pyrrhosoma nymphula* is the most abundant of 11 species of dragonfly which occur on the 89 hectare site, breeding in almost all of the 50 ponds and many ditches which occur on the mossland, and also in the woodland ponds (see TAYLOR, 1984).

At regular intervals through the study period, six samples of mature females were taken around both ponds. Between 10 and 20 females were caught on each occasion, either unpaired, in tandem, or beginning to oviposit. Females were caught as early in the day as possible to ensure that few eggs, if any, had been deposited. Captured females were taken back to the laboratory where their head widths were measured to the nearest 0.05 mm under a binocular microscope, and abdomen lengths were measured to the nearest 0.05 mm using dial callipers. Head width and abdomen length were significantly correlated ($r=0.729$, $n=94$, $P < 0.001$). Females were then killed by prolonged exposure to ether, and preserved in 70% alcohol until such times as they could be dissected.

Each female was dissected under 70% alcohol and the total number of mature eggs inside the abdomen was counted under a binocular microscope. Immature eggs of all sizes tended to occur in "strings" towards the anterior of the abdomen, and mature eggs could easily be distinguished from these by their larger size and darker colour. Thirty eggs were selected at random from the total for each female and measured to the nearest 0.01 mm under a binocular microscope fitted with an eyepiece micrometer. Finally, a sample of eggs was removed to be weighed. Using the tip of a scalpel blade, eggs were removed, one at a time, and placed in a drop of alcohol on a small piece of aluminium foil. The average number of eggs taken from each female was 79. The eggs were dried at 60° C for at least 7 days and were then dry weighed on a Cahn C29 electrobalance to the nearest 0.1 μg. The eggs and foil were first weighed, then the eggs were removed with a scalpel blade under a binocular microscope, and finally the foil was weighed to give the weight of the eggs by subtraction.

In the following analyses, mature egg numbers have been taken to be equivalent to clutch sizes. There were strong indications that almost all mature eggs were laid during an oviposition bout. Females caught towards the end of a day, after a natural separation from their partner, were found to possess low numbers of mature eggs relative to similar sized females caught earlier in the day (between 20-30 eggs compared with a mean of around 350 eggs for females caught early in the day; see also BANKS & THOMPSON, 1987a).

RESULTS

FACTORS AFFECTING EGG SIZE

The first sample of females was taken on 15 May and the last on 23 June. Over this period, mean egg weight ranged over 7.8 μg (from 11.6-19.4 μg, mean 14.3, SE 0.2, n=92). Egg length ranged over 0.18 mm (from 0.96-1.14 mm, mean 1.07, SE 0.01, n=95). There was no significant correlation between egg weight and egg length (r=0.056, n=92, P=0.594).

Initially, all data analyses were carried out separately for both measures of egg size. However, because identical conclusions could be drawn irrespective of which measure was used, only those using egg weight are presented. It was felt that, compared with a linear measurement, weight gave a more accurate indication of overall egg size in damselflies.

There was a significant decline in both head width and abdomen length of females over the study period (r=0.527, n=98, P < 0.001; and r=0.420, n=94, P < 0.001, respectively). Figure 1 shows the relationship between head width and sample date.

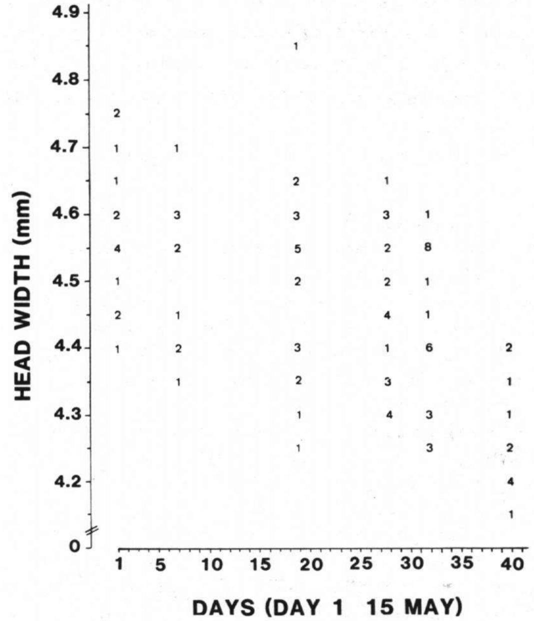

Fig. 1. The relationship between female head width and sample date; r=0.527, n=98, P < 0.001. The numbers indicate the number of females represented at each coordinate.

There was a significant

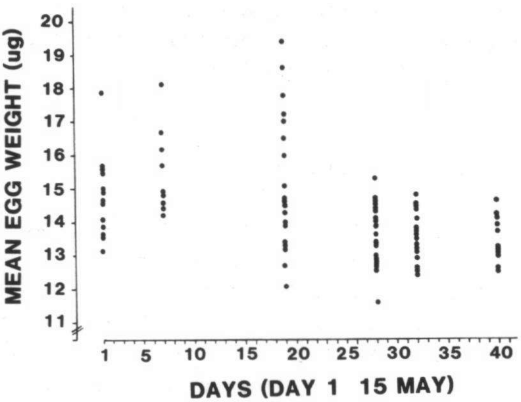

Fig. 2. The relationship between mean egg weight and sample date; r=0.448, n=92, P < 0.001.

decline in mean egg weight through the study period (Fig. 2), and a significant positive correlation between mean egg weight and female head width (Fig. 3), but not abdomen length (r=0.103, n=90, P=0.336). However, one or other of the relationships in Figures 2 and 3 most likely arose beacuse of intercorrelations between egg weight, female head width, and sample date, thereby necessitating partial correlation coefficients to be calculated, controlling for head width and date respectively. The relationship between mean egg weight and female head width was lost when date was controlled for (r=0.017, n=87, P=0.878), while the relationship between mean egg weight and sample date remained significant when head width (or abdomen length) was controlled for (r=0.388, n=87, P < 0.001).

There was a significant positive correlation between mean egg weight and clutch size (r=0.311, n=92, P < 0.01). However, both egg weight and clutch size (see below) declined with date of sampling, and spurious correlations were generated when either variable was plotted against skeletal size measures. When the complicating effects of date and female size were held constant using partial correlation analyses, the significant relationship between mean egg weight and clutch size was lost (partial correlation controlling for sample date, r=0.174, n=89, P=0.098; partial correlation controlling for sample date and head width, r=0.176, n=88, P=0.098).

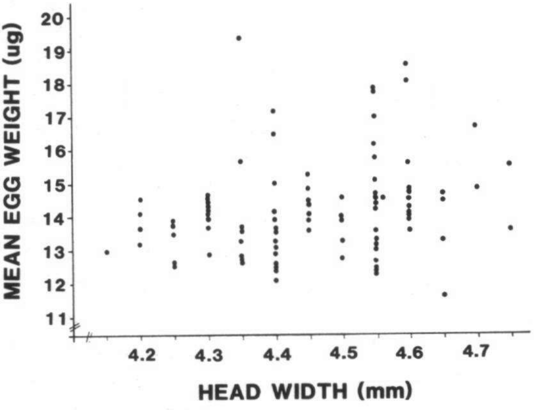

Fig. 3. The relationship between mean egg weight and head width of females; r=0.250, n=92, P < 0.05.

The conclusions of the correlation analyses were supported by a multiple regression analysis which showed that the date on which a sample of females was caught was the only significant variable accounting for variation in egg weight ($F_{1,90}=22.64$, $P < 0.001$; sample date contributed to 20.1% of the total variation in mean egg weight).

FACTORS AFFECTING CLUTCH SIZE

Egg numbers dissected from a total of 95 females ranged from 68-754 with a mean of 350.6 (SE 14.5). There was a significant negative correlation between clutch size and sample date (Fig. 4), and a significant positive correlation between clutch size and female head width (Fig. 5). As with egg weight, it was necessary to calculate partial correlation coefficients to determine which of the two relationships was genuine. The correlation between clutch size and female head width was lost when sample date was controlled for ($r=0.185$, $n=90$, $P=0.078$), but when head width was held constant, clutch size and date remained significantly correlated ($r=0.217$, $n=90$, $P < 0.05$). Multiple regression analysis also indicated that sample date was the only significant variable accounting for variation in clutch size ($F_{1,90}=14.45$, $P < 0.001$; sample date explained 13.8% of the total variation in egg numbers).

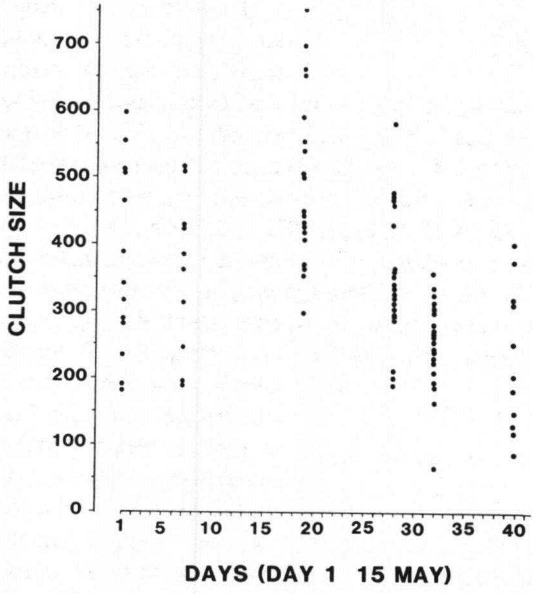

Fig. 4. The relationship between clutch size and sample date; $r=0.364$, $n=95$, $P < 0.001$.

To determine whether the physical size of a female's abdomen limited the maximum number of eggs in a clutch, clutch sizes were plotted against abdomen lengths of all females sampled. The correlation between the two variables was positive and significant ($r=0.299$, $n=93$, $P < 0.01$), but disappeared when the effects of sample date were controlled for ($r=0.175$, $n=90$, $P=0.099$). However, abdomen size may only exert a limiting effect when clutch sizes are particularly high. To test this, females having a clutch size of less than 400 eggs were excluded from analyses. As before, a significant positive correlation was found between clutch size and abdomen length ($r=0.41.4$, $n=32$, $P < 0.05$), but this relationship remained significant when a partial correlation coefficient was calculated controlling for sample date ($r=0.403$, $n=29$, $P < 0.05$).

There were indications, albeit from only qualitative observations, that the decline in clutch size over the study period was influenced by the pattern of sunny and cloudy days prior to the sampling date (and therefore the inter-clutch interval, since females only engage in reproductive activity on fine days). For instance, the third sampling occasion (day 19 or 2 June) followed four days of overcast, dull weather on which there was little or no reproductive activity. Egg numbers at this time were higher than might have been expected (see Fig. 4). Females unable to visit the ponds over this period because of overcast weather would presumably have been able to mature large clutches of eggs. By contrast, towards the end of the study period, weather conditions were warm, sunny and suitable for reproductive activity and clutch sizes fell towards the lower end of the range.

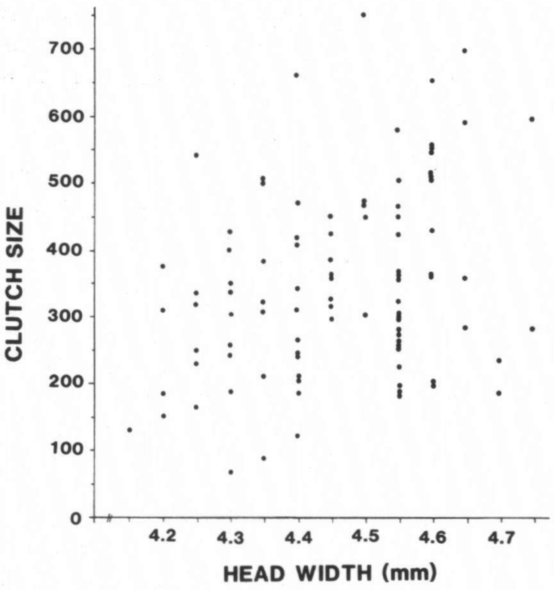

Fig. 5. The relationship between clutch size and head width of females; $r=0.327$, $n=95$, $P < 0.01$.

If variation in clutch size arose predominantly because of differences in the inter-clutch interval, it would suggest that the negative relationship found between clutch size and sampling date could equally as well have been positive or non-significant, given different weather patterns during the study period.

DISCUSSION

Despite the large amount of variation in mean egg weight (range 11.6-19.4 μg), no relationship was found between egg weight and female size, when controlling for the effects of sample date. Similarly, clutch size was not correlated with female head width when sample date was controlled for, although there were indications that, for large clutches, abdomen size and clutch size were significantly positively correlated. That such a relationship between clutch size and female skeletal size was only evident when egg numbers were high suggests that the maximum clutch size may be limited by the physical space inside a female's abdomen, rather than, for instance, larger females being more successful in competition for food.

In a study of *Coenagrion puella,* BANKS & THOMPSON (1987a) also found that mean egg weight was not correlated with female skeletal size. However, they found an inverse relationship between female size and clutch size for those clutches taking one day to mature (the majority of clutches), and they suggested that clutch size would be a decreasing function of body size because of the way in which the energetic cost of flight, and other metabolic costs, scaled with body size. There was no such relationship in clutches taking longer than one day to mature, and they found that overall, lifetime egg production was positively correlated with female body size since larger females produced a greater number of clutches.

Clutch size was found to vary between 68-754 eggs (mean 350.6), despite females being caught at the very beginning of a day's reproductive activity. Female *Coenagrion puella* contained an average of 12.7 eggs as they flew away from a breeding site at the end of oviposition, whereas initial clutch sizes ranged between 110 and 250 eggs (BANKS & THOMPSON, 1987a). In a study of *Chromagrion conditum,* BICK et al. (1976) found that eggs were deposited at a rate of 5.5 per minute, resulting in an expected average complement of 200 eggs being deposited for each sequence of oviposition. WAAGE (1978) found that egg deposition rates for *Calopteryx maculata* averaged 7-10 eggs per minute in four common plant species. Multiple bouts for marked females showed that an average of 230-328 eggs may be laid per day, while maximum duration of oviposition per day was 75 minutes which would correspond to between 525-700 eggs being deposited. Consequently, it is likely that even when egg numbers were particularly high almost all would be laid during a day's oviposition by female *P. nymphula.*

Both mean egg weight and clutch size were found to be significantly negatively correlated with sample date. That is, females visiting the ponds later in the season laid smaller clutches and smaller eggs than those arriving early in the season, irrespective of size. There are several possible explanations for this. If the differences in clutch size and egg size between females are related to differences in individual body condition, it is possible that late-arriving females, and therefore late-emerging females (see below), may have suffered either from the detrimental effects of asymmetric competition as larvae, or from a shorter maturation period relative to early-emerging females. Alternatively, the decline in clutch size and egg size may be unrelated to body condition, but closely associated with the pattern of sunny and cloudy days over the study period, and therefore the inter-clutch interval.

The reproductive lifespan of female damselflies is likely to last only a few days.

CORBET (1952) estimated the average lifespan of adult *P. nymphula* to be 6.76 days. Recalculating Corbet's original data, the mean reproductive lifespan for males was 7.01 days, while for females it was 5.50 days. Therefore, those individuals caught later in the season were likely to have emerged later and reached sexual maturity later than those females caught early on. The relationship between larval growth rate, development rate and feeding rates has been described by LAWTON et al. (1980) and PICKUP & THOMPSON (1990) for damselflies; poorly fed larvae take longer to develop through each instar and give rise to smaller adults. Thus, late-emerging females may have suffered reduced development rates and size increases as aquatic larvae, resulting from competition with larger, early-emerging adults (see BANKS & THOMPSON, 1987b; GRIBBIN & THOMPSON, 1990). In a laboratory study of *P. nymphula* larvae, HARVEY & CORBET (1985) showed that size in the final instar was positively correlated with the provision of food during the last 3 or 4 instars, and that larval and adult size were positively correlated. If the detrimental effects of asymmetric competition were to affect directly a female's egg producing capability, this might explain why late-emerging females laid smaller clutches of smaller eggs compared to early-emerging females.

There may be considerable selective pressure on females to initiate reproduction early in the breeding season, or when reproductive activity is reaching a peak, such that they have access to a large number of potential mates, and such that their eggs are laid and hatch relatively early in the summer. Larvae of those adults that are present at the end of the breeding season will be smaller than other larvae in their year class and may suffer from interference competition which could exacerbate differences in size and development rate (GRIBBIN & THOMPSON, 1990). Consequently, there may be a tradeoff between rapid maturation and full reproductive development and/or egg production for late-emerging females of *P. nymphula*.

Although over a thousand females were caught and marked at emergence or when mature, the low recapture rate of marked individuals, resulting either from dispersal or mortality, prevented accurate data on longevity, and therefore information on lifetime reproductive success, being collected. However, longevity is unlikely to be a major factor explaining the decline in mean egg weight and clutch size through the study period since the mean reproductive lifespan of female *P. nymphula* is short relative to the total study period over which samples were collected (5.50 days and 40 days respectively).

Sample date may affect clutch size via the pattern of sunny and cloudy days over the period of reproductive activity (and thus the time since females last oviposited or the interclutch interval). BANKS & THOMPSON (1987a) found that the most important determinant of clutch size in *Coenagrion puella* was the time since the previous clutch was laid. They found that the longer the inter-clutch interval, the larger the subsequent clutch, with the maximum being reached after

3-4 days. However, females would maximize their lifetime egg production by minimizing the period between clutches. This is because a clutch that took two days to mature was less than twice as big as one that took one day to mature, and because the shorter the period between clutches, the higher the probability of surviving to lay another clutch (see also THOMPSON, 1990).

There were indications that female *P. nymphula* may not have visited the ponds on every day to breed. During a period in which over 85% of days were sunny, most (60%) clutches of *C. puella* were produced on the day following the previous clutch (BANKS & THOMPSON, 1987a). The mean weight of newly emerged females was 39.2 mg while the mean weight of mature females was 50.5 mg, the range in weight of mature females being 33.2 mg (M.J. Banks, unpublished). For female *P. nymphula* the mean weight at emergence was 39.8 mg and the mean weight of mature females was 66.1 mg, the range in weight of mature females being 45.7 mg (S.D. Gribbin, unpublished). Thus female *C. puella* show a 28.8% increase in original mean wet weight from emergence to maturity, while female *P. nymphula* show a 66.1% increase in original mean weight. Such a substantial difference in weight increase between the two coenagrionids might suggest that female *P. nymphula* build up large clutches of eggs over comparatively long inter-clutch intervals, and thus visit the ponds only on occasions. Females might be prevented from reproducing due to adverse weather conditions, or they might actively avoid the pond, even on fine days.

FINCKE (1988) found that female *Enallagma hageni* visited a study pond on average once every 6 days. Female *E. hageni* submerge completely to oviposit and suffer a fixed probability of failing to escape from the water at the end of each bout of ovipositing, which is independent of the duration of the oviposition bout. They minimize the risk of mortality by minimizing the number of oviposition bouts, and thus by producing large clutches with longer inter-clutch intervals (O.M. Fincke, personal communication with BANKS & THOMPSON, 1987a). In this study *P. nymphula* females were at risk from predation from water beetles, dragonfly larvae, and frogs and newts during an oviposition bout, and it is possible that they might reduce the risk of mortality by reducing their total number of oviposition bouts. However, the probability of predation is likely to increase with the duration of oviposition, and BANKS & THOMPSON (1987a) indicated that a higher risk of mortality during oviposition would not favour longer inter-clutch intervals unless the rate of egg laying during oviposition increased with the inter-clutch interval.

To conclude, several explanations have been proposed to account for the decline in egg size and clutch size through the season, and there are indications that climatological factors have the potential to exert a strong effect on the reproductive biology of female *P. nymphula*. Not only will bad weather prevent reproductive activity, but it is also highly likely that egg production is related to temperature. There is clearly a need for more detailed studies to investigate further those factors affecting egg size, clutch size and lifetime reproductive success in *P. nymphula,* and, more generally, the reproductive biology and behaviour of other female odonates.

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