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QUANTITATIVE ASPECTS OF THE MORPHOLOGY AND REPRODUCTIVE BIOLOGY OF *NEPA TRINACRIAE* MAZZA (RHYNCHOTA HETEROPTERA)

Riassunto — *Aspetti quantitativi della morfologia e della biologia riproduttiva di* Nepa trinacriae *Mazza (Rhynchota Heteroptera). Nepa trinacriae* Mazza è una specie di recente istituzione (MAZZA, 1981) molto diffusa nell'isola di Sicilia. In base alle caratteristiche morfologiche tradizionalmente usate nella sistematica dei Nepidi, è stata erroneamente classificata come *Nepa rubra* L. (STICHEL, 1955), *Nepa rubra minor* Put. (POISSON, 1957), *Nepa seurati* Bergv. (SEIDENSTÜCHER, 1963; SERVADEI, 1967) ed infine come *Nepa cinerea seurati* Bergv. (TAMANINI, 1973, 1979). Particolari aspetti della biologia riproduttiva e della morfologia di *Nepa trinacriae* sono stati presi in esame per portare un contributo ad una migliore conoscenza della specie.

Abstract — The results of observations made on the morphology and reproductive biology of *Nepa trinacriae* are reported in order to provide a better understanding of the nature of this species.

Key words — Egg biology, water scorpions, *Nepa trinacriae*.

INTRODUCTION

The fresh water scorpions of Italy belong to the small yet widely distributed family of the Nepidae. They are found, up to 1500 m above sea level, in slow-flowing or stagnant waters and along the banks of meandering streams or irrigation canals. They usually hide at the waters-edge underneath stones or in the mud and vegetation. Along with other freshwater insects, these organisms are vulnerable to urban or agricultural expansion which often involves draining ponds and marshes. The water scorpions

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that are found in Italy include *Nepa cinerea* Linneo, *Nepa sardiniensis* Hungerford and *Nepa trinacriae* Mazza. They have different distributions and population densities (Fig. 1).

Fig. 1 - Geographical distribution of the currently known species of *Nepa* in Italy.

The well-known *Nepa cinerea* is present in many areas of the Palearctic region including the Italian peninsula. At present, the other known species of Italian water scorpions are *Nepa sardiniensis,* found throughout the Island of Sardinia, with a low population density, and *Nepa trinacriae,* found throughout the Island of Sicily, with a high population density.

The morphological characteristics traditionally used by authors to classify different species of Nepidae are of no avail if we wish to distinguish *Nepa trinacriae* from other Italian water scorpions, even though the latter are reproductively isolated from the former (MAZZA, 1981). Thus the water scorpions of Sicily have been wrongly attributed to various species or subspecies including *Nepa rubra* L. (STICHEL, 1955), *Nepa rubra minor* Put. (POISSON, 1957), *Nepa seurati* Bergv. (SEIDENSTÜCHER, 1963; SERVADEI, 1967) and, recently, to *Nepa cinerea seurati* Bergv. (TAMANINI, 1973, 1979).

In order to provide a better understanding of the nature of *Nepa trinacriae,* this paper will present the result of observations made on the morphology and reproductive biology of *Nepa trinacriae* specimens which were originally collected in the field and then reared in the laboratory between 1978 and 1983.

Material and methods

The various specimens of *Nepa trinacriae* used for this study were collected from northern, central and southern sites located along the Ionian coast of Sicily, that is on the Alcantara River, along an irrigation canal at the southern boundary of Catania and on the Asinaro River near Noto.

The specimens were transferred to the laboratory where they reproduced, were isolated in pairs and kept in 300 cc containers filled with 100 cc of water and green filamentous algae. The water was changed daily. The specimens were subjected to a natural photoperiod, unless otherwise indicated. Initially, they were reared for 24 months at an ambient temperature ranging between 14° and 30°C protected from direct sunlight in a room facing south. In the next 24 months they were kept in a room facing north with an ambient temperature ranging between 8° and 30°C. Eggs were counted and removed either daily or every few days. Newly hatched larva were fed *Daphnia.* Larvae at later stages of development and adults were fed *Asellus* or *Chironomus* larvae. Specimen dimensions were calculated by means of a Wild M5 microscope and a 10 X ocular micrometer and are expressed in «work units» depending on the lens used. Thus, for lenses with magnification powers of 6, 12, 25 and 50 times real size, the corresponding work units are WU6, WU12, WU25 and WU50 which are equivalent to 1/6, 1/12, 1/25 and 1/50 mm, respectively. Throughout the text and in the tables, means are followed by ± the standard deviation.

Observations and results

Italian Nepidae are anautogenous Rhynchota that are well adapted to the environment in which they live. They must stay in

fresh water for all their life, even during embryonic development inside their eggs. Their body is flattened to offer low resistance to water currents. Their surface area, in relation to their volume, is very large and permits the formation of an extensive respiratory plastron located under the hemelytra. If these insects are left out of water even for brief periods of time, then dehydration and death rapidly occur because of their extensive surface area. They have lost the ability to fly and have developed certain organs which prevent the fore wings from separating (MAZZA, 1974). These organs are located at the overlapping membranous regions of the hemelytra.

Even if they were never inseminated but if they are adequately nourished, adult females can mature and deposit eggs provided the necessary conditions of temperature, photoperiod and substrate are present.

Each ovary of the water scorpion consists of five ovarioles the structure of which is typical of the other telotrophic ovarioles found in Rhynchota (HAMILTON, 1931; BONHANG, 1958). The ovarioles are fixed to the dorsal edge of the thorax by the terminal filament and are suspended in the hemocoel by branches of the second abdominal spiracular tracheae.

Three major developmental stages may be distinguished in the oocyte: 1) previtellogenesis 2) vitellogenesis and 3) chorion secretion. In a given water scorpion, all the ovarioles may be essentially identical in relation to the number of oocytes and oocyte stages. In several cases, however, there is heterogeneity both in the size of the terminal oocyte and in the number of ovulated eggs. Inside a given ovary, certain terminal oocytes might be in the previtellogenic stage while others are in various intermediate stages of vitellogenesis.

Each ovariole has a long pedicel where several ovulated eggs may accumulate simultaneously. At the sixth abdominal segment the pedicels join to form two short oviducts. These, in their turn, join to form a muscular vagina. Two diverticula protrude, one behind the other, from the dorsal wall of the vagina. The anterior diverticulum, the spermatheca, is a long blind-ending coiled tube. The posterior one is a flattened lobe believed to be an accessory gland but its structure and function have never been studied.

Female water scorpions lay their eggs inserting them in water vegetation. Thus the eggs are generally found in a floating

mass of filamentous green algae that are often intermixed with the floating leaves of certain species of *Potamogeton, Lemna, Callitriche,* or the roots of *Nasturtium officinalis* and *Apium nodiflorum,* to form a green mat of vegetation on the surface of stagnant or slow-flowing water. In running water, eggs that are glued to vegetation growing along the shoreline, can occasionally be immersed under water for variable periods of time. In such circumstances, embryonic development occurs, but at a slower rate.

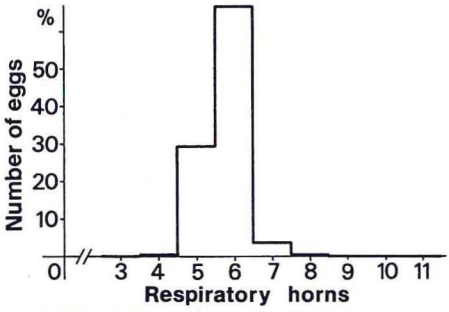
The eggs of Nepidae have a rather complex structure and morphology. Various authors (LEUCKART, 1855; KORSCHELT, 1884; KORSCHELT and HEIDER, 1902; POISSON, 1933; HINTON, 1961, 1962; COBBEN, 1968; OGORZALEK, 1974) have described a posterior-dorsal hydropylar region, an anterior-dorsal micropylar region and a hatching operculum at the anterior pole. Around the operculum is a crown of long appendages called respiratory tubules or respiratory horns. These appendages are produced by follicular cells which undergo hypertrophy after the oocyte nucleus moves to the apical-lateral portion of ooplasm facing them. There are seven hypertrophic follicular cells in *Nepa cinerea* and two in *Ranatra linearis* (KORSCHELT, 1887; OGORZALEK, 1974). In *Ranatra linearis,* the respiratory horns and follicular cells which originate them, are always two in number (MAZZA, 1978).

In contrast, in the different species of *Nepa,* the number of respiratory horns are always variable; whether there is corresponding variation in follicular cells is not known. The precise range of variability has been previously found in *Nepa cinerea* and *Nepa sardiniensis* (MAZZA, 1968) and was used to study the possible mechanism of hereditary transmission. All possible crosses made between males and females born from eggs with the same or a different number of respiratory horns, never gave rise to females that produced eggs which had a single given number of respiratory horns. The range of variability, however, could change under the influence of hereditary factors and the number of respiratory horns could fluctuate under the influence of environmental factors (MAZZA, 1978).

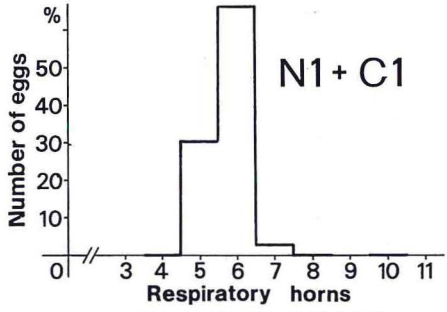
The Number of Respiratory Horns

The number of respiratory horns was observed in 25154 eggs laid by 88 female specimens of *Nepa trinacriae,* from nature or

reared in the laboratory, during a 52 month period. The number of respiratory horns varied from 3 to 11 (Fig. 2. Left).



3	4	5	6	7	8	9	10	11	Tot eggs
1	33	7371	16721	991	29	6	1	1	25154



3	4	5	6	7	8	9	10	11	Tot eggs
	6	2613	5636	250	8		1		8514

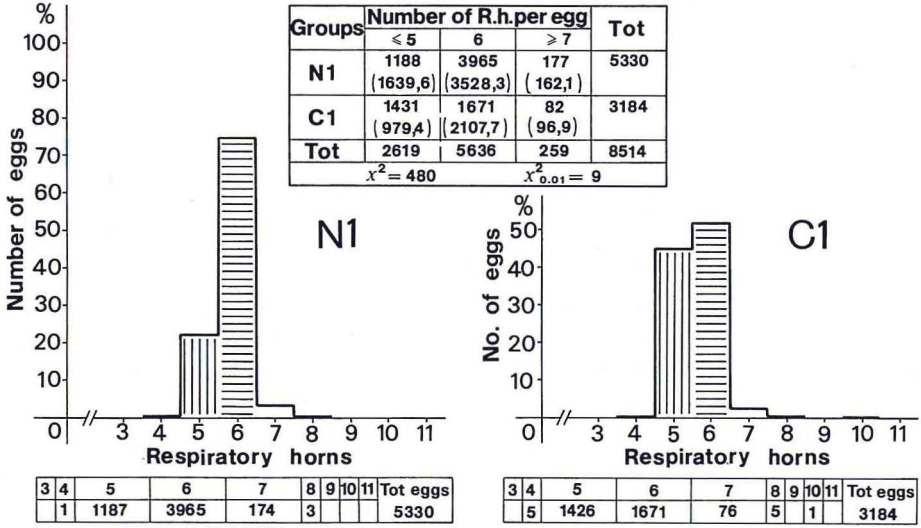
Fig. 2. Left - Histogram and frequency table of the number of respiratory horns observed in 25154 eggs laid by 88 female *Nepa trinacriae* reared over a period of 52 months between August 1978 and January 1983.
Right - Histogram and frequency table of the number of respiratory horns of the combined N1 and C1 groups of 8514 eggs laid throughout the lifetimes of 26 females belonging to two different samples of *Nepa trinacriae* gathered near Noto and Catania, respectively.

The least frequent variants, those with 3 or with 11 respiratory horns, might be the result of pathologic alterations of the ovarioles or of external environmental conditions. A strong thermic shock, for instance, was shown to cause an increase in the number of respiratory horns towards the top of the range of variability (unpublished results).

There were relatively few eggs with 4, 7, 8, 9, or 10 respiratory horns and those with 6 were the most numerous. These latter eggs represented 66.5% of all the eggs examined but, for a given female, they did not necessarily represent the majority of eggs produced. For instance, eggs with 5 respiratory horns represented only 29.3% of all the eggs examined, but, in certain cases, they represented the most common type of egg laid by a given female.

The fact that females reared in the same laboratory conditions produce a majority of eggs with 5 or 6 respiratory horns does not seem to be a random event but seems rather to be associated with factors that are transmitted from generation to gene-

ration. In the two initial samples, N1 and C1, of eggs that were laid in the laboratory by two groups of females which were collected near Noto and Catania, respectively, different characteristics were found and they were also maintained in samples of eggs produced by females of successive generations born in the laboratory and reared under similar laboratory conditions. The frequency of eggs with 5 or 6 respiratory horns in the combined N1 and C1 groups does not significantly differ from the overall frequency of the entire group of eggs studied (Fig. 2. Right). However, when N1 and C1 are compared to each other, one can see that these groups are not homogeneous (Fig. 3). In particular, N1 is characterized by a low frequency of eggs with 5 respiratory horns (22.3%) and a high frequency of eggs with 6 respiratory horns (74.4%) while, for C1, there are 44.8% eggs with 5 respiratory horns and 52.5% with six. Figure 3 contains, at the top, a contingency table and the Chi squared value to test the homoge-



Groups	Number of R.h.per egg ⩽5	6	⩾7	Tot
N1	1188 (1639,6)	3965 (3528,3)	177 (162,1)	5330
C1	1431 (979,4)	1671 (2107,7)	82 (96,9)	3184
Tot	2619	5636	259	8514

$x^2 = 480$ $x^2_{0.01} = 9$

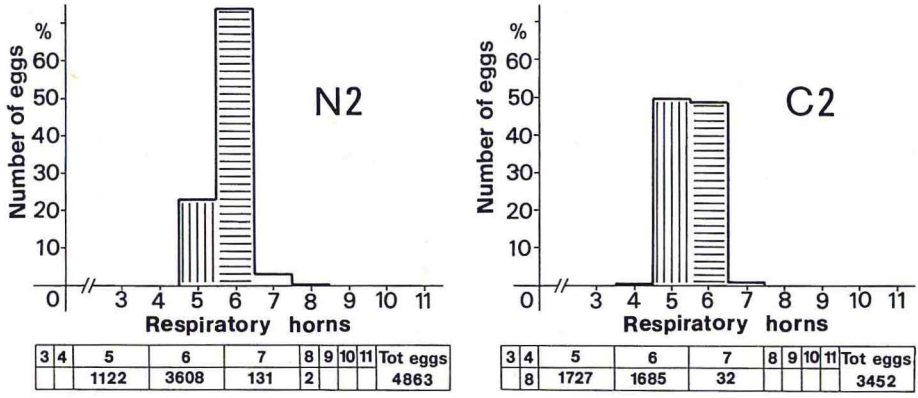
3	4	5	6	7	8	9	10	11	Tot eggs
	1	1187	3965	174	3				5330

3	4	5	6	7	8	9	10	11	Tot eggs
	5	1426	1671	76	5		1		3184

Fig. 3. Left - Histogram and frequency table of the number of respiratory horns present in the N1 group of 5330 eggs laid throughout the lifetimes of 14 female *Nepa trinacriae* collected in the larval or adult stages near Noto.
Right - Histogram and frequency table of the number of respiratory horns present in the C1 group of 3184 eggs laid throughout the lifetimes of 12 female *Nepa trinacriae* collected in the larval or adult stages near Catania. Both groups were compared to each other and not found to be homogeneous. At the top is a table showing the calculated Chi squared value.

neity of the N1 and C1 samples. It also contains, on the left, a histogram and a frequency table of the number of respiratory horns of 5330 eggs (group N1) laid in the laboratory by 14 females that were collected near Noto and, on the right, a histogram and a frequency table of the number of respiratory horns of 3184 eggs (group C1) laid by 12 females collected near Catania.

Successive generations, within the same sample, produced eggs that maintained a different distribution frequency of respiratory horns. The results of the second (N2 and C2) and third (N3 and C3) generations grown in identical laboratory conditions are shown in figures 4 and 5, respectively.

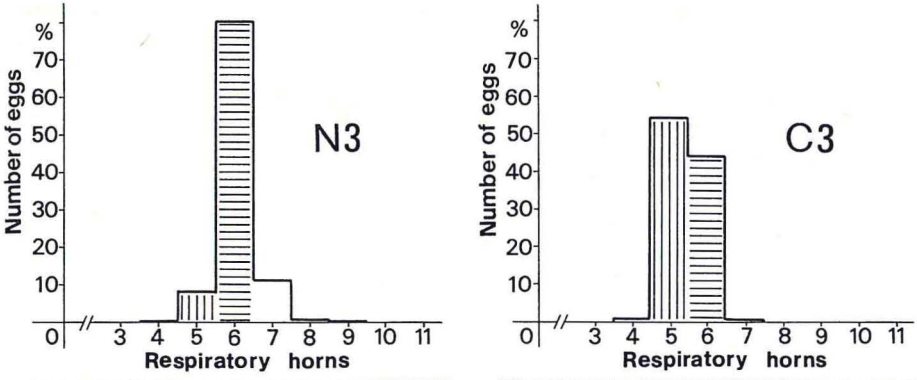


3	4	5	6	7	8	9	10	11	Tot eggs
		1122	3608	131	2				4863

3	4	5	6	7	8	9	10	11	Tot eggs
	8	1727	1685	32					3452

Fig. 4. Left - Histogram and frequency table of the number of respiratory horns present in the N2 group of 4863 eggs laid throughout the lifetimes of 16 female *Nepa trinacriae* constituting generation 2 of the Noto sample.
Right - Histogram and frequency table of the number of respiratory horns present in the C2 group of 3452 eggs laid throughout the lifetimes of 6 female *Nepa trinacriae* constituting generation 2 of the Catania sample. Groups N2 and C2 are not homogeneous.

The differences observed in successive generations of *Nepa trinacriae* from Noto and Catania, can also be seen by comparing the frequency distribution of the females of the entire N sample, characterized by percentages of eggs with 5 respiratory horns or less laid throughout their lifetimes, and the frequency distribution of the females of the entire C sample characterized by percentages of eggs with 5 respiratory horns or less laid throughout their lifetimes (Fig. 6).



3	4	5	6	7	8	9	10	11	Tot eggs
	2	319	3207	440	14	6			3988

3	4	5	6	7	8	9	10	11	Tot eggs
	12	962	778	11				1	1764

Fig. 5. Left - Histogram and frequency table of the number of respiratory horns present in the N3 group of 3988 eggs laid throughout the lifetimes of 17 female *Nepa trinacriae* constituting generation 3 of the Noto sample.
Right - Histogram and frequency table of the number of respiratory horns present in the C3 group of 1764 eggs laid throughout the lifetimes of 7 female *Nepa trinacriae* constituting generation 3 of the Catania sample. Groups N3 and C3 are not homogeneous.

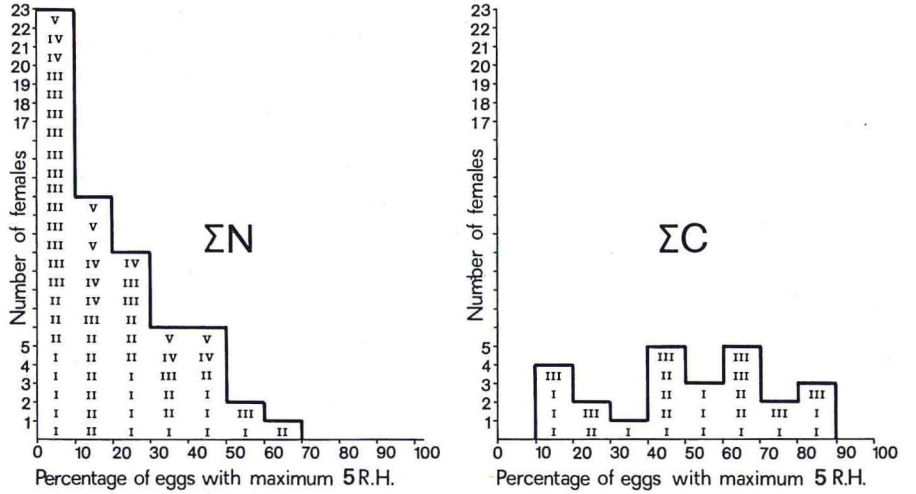


Fig. 6. Left - Frequency distribution histogram of 61 female *Nepa trinacriae*, from five generations (I, II, III, IV, V) of the whole N sample, plotted against the percentage of eggs, bearing 5 or less respiratory horns, laid by each of these females throughout their lifetime.
Right - Frequency distribution histogram of 25 female *Nepa trinacriae*, from three generations (I, II, III) of the total C sample, plotted against the percentage of eggs, bearing 5 or less respiratory horns, laid by each of these females throughout their lifetime. The two samples are statistically different.

The Size of Eggs

The dimensions of *Nepa trinacriae* eggs are characteristics that distinguish this species from other Italian *Nepa.* Figure 7, on the left, shows how the size of the eggs, just laid, was calculated. Two or three respiratory horns were measured in both the dorsal and the ventral regions of the egg. To be more precise, three respiratory horns were measured when there were respiratory horns situated in the plane of the bilateral symmetry of the egg and two respiratory horns were measured when this was not the case (Fig. 7. Right).

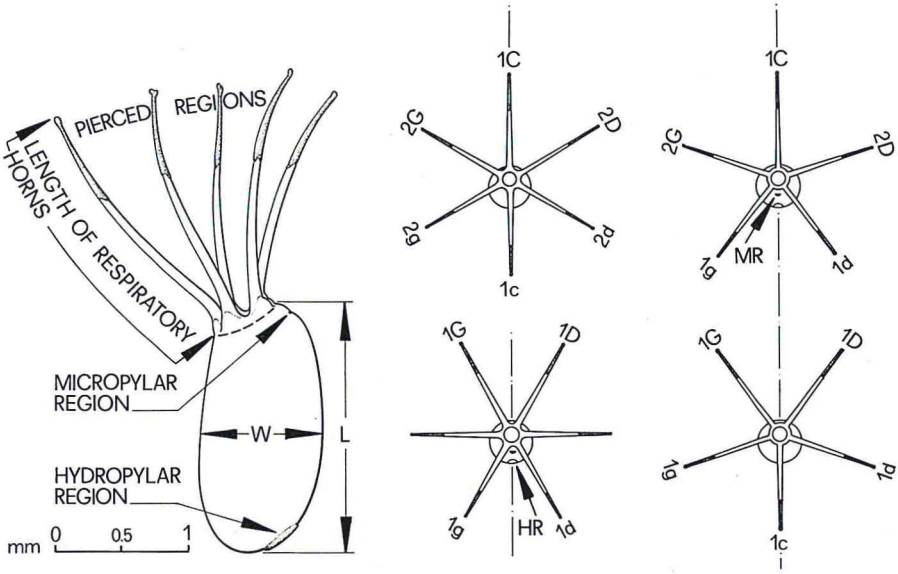


Fig. 7. Left - Side view of a *Nepa trinacriae* egg to show how measurements of the egg shell, the micropylar region, the respiratory horns and their pierced regions were taken.
Right - Sketch of *Nepa trinacriae* eggs seen from above. The respiratory horns can lie in various positions in relation to the plane of bilateral symmetry of the egg. Their position is indicated by a number and a letter.

Table 1 contains the measurements of shell length and width, the micropylar region, the respiratory horns and their pierced regions.

A statistical analysis of the respiratory horns showed that their size varied according to their position (Table 2) and that

TABLE 1 - *Size of newly-laid eggs of* Nepa trinacriae.
L = length of shell; W = width of shell; Mr = length of the micropylar region; 1C, 1G, 2G, 1D, 2D, 1c, 1g, 2g, 1d, 2d = length of respiratory horns in the positions indicated; pr1C, pr1G, pr2G, pr1D, pr2D, pr1c, pr1g, pr2g. pr1d, pr2d = length of pierced regions; N = total number of measurements; M = means; S = standard deviation. The measurement are in millimetres.

Size of Eggs	N	M	ΣX^2	S
L	250	1.8592	865.55	0.074786
W	250	0.92864	215.96	0.038136
Mr	180	0.088611	1.4136	0.018872
1C	105	1.8238	350.60	0.11338
pr1C	105	0.63724	41.545	0.047566
1(G,D)	290	1.8054	950.09	0.12884
pr1(G,D)	290	0.61834	111.68	0.05264
2(G,D)	210	1.7710	661.53	0.11660
pr2(G,D)	210	0.63486	85.136	0.048757
1c	110	1.6275	292.88	0.11858
pr1c	110	0.65654	47.769	0.056947
1(g,d)	280	1.6487	764.84	0.11557
pr1(g,d)	280	0.64971	119.08	0.056441
2(g,d)	220	1.7018	640.14	0.11667
pr2(g,d)	220	0.66018	96.571	0.055084

their diameter got smaller as their total number increased. The difference between the mean length of the longest or ventro-medial respiratory horns and the shortest or dorso-medial respiratory horns was 0.1973 mm ± 0.0001.

The Number of Eggs

The reproductive capacity of *Nepa trinacriae* was examined in laboratory conditions. No data concerning the reproductive poten-

TABLE 2 - *Analysis of variance for the data in table 1.*
V.R.H. = ventral respiratory horns; D.R.H. = dorsal respiratory horns; D.F. = degrees of freedom.

Source of variation	D.F.	Sum of squares	Mean square	f ratio
Comparison between lenghts of V.R.H.				
1C → 1(G,D)+2(G,D)	1	0.0934	0.0934	6.26
1(G,D) → 2(G,D)	1	0.14412	0.14412	9.67
Error	602	8.9758	0.014910	
Total	604	9.2134		
Comparison between lenghts of D.R.H.				
1c → 1(g,d)+2(g,d)	1	0.017956	0.17956	13.2
1(g,d) → 2(g,d)	1	0.34740	0.34740	25.6
Error	607	8.2404	0.013576	
Total	609	8.7673		

tial of other Nepidae is available. However, *Nepa trinacriae* appear to have a high reproductive potential compared to other well known Heteroptera (ENGELMANN, 1970; HINTON, 1981).

Table 3 contains comprehensive data concerning eggs laid by 88 female *Nepa trinacriae* reared in the laboratory over a 52 month period between August 1978 and January 1983. The table also gives the average egg production in a given month over successive years, the overall average of monthly egg-laying and other statistical parameters.

The reproductive capacity of the females of this species varies greatly. Figure 8 shows the length of the period of reproductive activity and the number of eggs laid during that period by each one of 70 female *Nepa trinacriae* that were given 1 cc of *Asellus* per female once or twice a week and reared in the same

TABLE 3 - *Data regarding 25154 eggs laid by 88* Nepa trinacriae *females reared in the laboratory over a 52 month period between August 1978 and January 1983.*
N = overall number of female presences each month over a 52 month period. ΣX = number of eggs: M = mean number of eggs per female; S = standard deviation.

	N	ΣX	ΣX^2	M	S
JAN	113	1129	30327	9.9911	13.041
FEB	124	1966	53976	15.855	13.617
MAR	130	2669	85345	20.531	15.389
APR	132	2513	95171	19.038	19.008
MAY	135	2639	139989	19.548	25.685
JUNE	129	2938	139778	22.775	23.859
JULY	120	2938	158414	24.483	26.958
AUG	114	2080	105746	18.246	24.495
SEP	123	2477	112935	20.138	22.734
OCT	128	1889	56403	14.758	14.987
NOV	128	1053	23097	8.2266	10.661
DEC	121	863	15065	7.1322	8.6168
TOT.	1497	25154	1016246	16.803	19.919

laboratory conditions. For the sake of simplicity, the period of reproductive activity was considered to be the interval between the first and last oviposition. In the specimens studied, this period lasted an average of about 20 months and varied from a minimum of 5 to a maximum of 32 months (Fig. 8. Left).

The total number of eggs laid during the entire reproductive life of a female varied from 50 to about 800 eggs with an average of 325 (Fig. 8. Right).

Factors that Affect Egg Production

The number of eggs that can both mature and be laid varies from one individual female to another and also depends on tem-

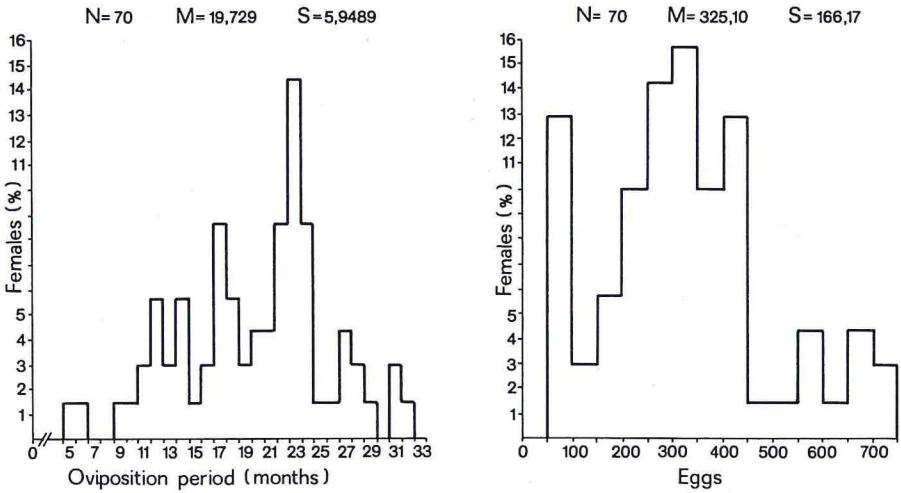


Fig. 8. Left - Histogram showing the frequencies of the length of the oviposition period of a group of 70 female *Nepa trinacriae* given 1 cc of *Asellus* per female once or twice a week and reared in the same laboratory conditions. N = number of females; M = mean of the period of reproductive activity; S = standard deviation.
Right - Histogram showing the frequencies of the number of eggs laid throughout the reproductive life of each of 70 female *Nepa trinacriae* reared in the same laboratory conditions. N = number of females; M = mean of eggs per female; S = standard deviation.

perature, photoperiod, the presence of suitable vegetation for oviposition and, most important, on the amount of food ingested by the females. Several female *Nepa trinacriae* were reared in a room facing south and were given 1 cc/female of *Asellus* once or twice a week. Data gathered from these specimens during the first 28 months of laboratory observation showed that the threshold of reaction to various environmental stimuli was not the same for all females and that there was a correlation between the number of females that laid eggs and the mean number of eggs that each female laid ($r_{obs} = 0.47$, $r_{0.975} = 0.38$). The results from each of the 28 months of observation are summarized in Fig. 9. In particular, the following parameters are given: the mean maximum and minimum room temperatures, the percentage of egg-laying females, the average number of eggs laid per female, the 95% confidence limits and corresponding confidence intervals. The data show that when environmental conditions in the laboratory (temperature, light, amount of food) vary, then the mean

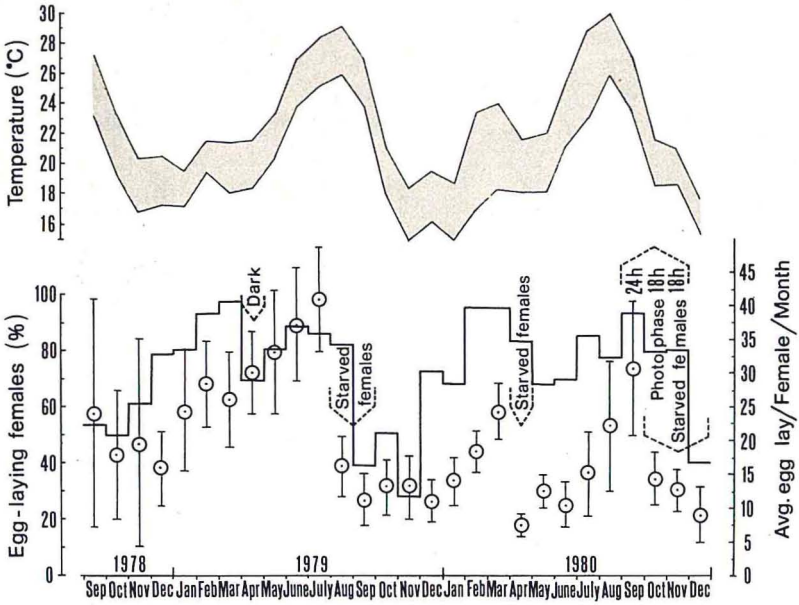


Fig. 9 - Correlation between the percentage of egg-laying females and the mean number of eggs per female laid during a 28 month observation period (r = 0.47). Data for each month include: average maximum and minimum temperatures (upper graph); the percentage of egg-laying females (histogram); the average number of eggs laid (dotted circles), the 95% confidence limits and corresponding confidence intervals. The specimens of *Nepa trinacriae* that were utilized for these observations, were reared in a room facing south. Unless otherwise indicated, they were given 1 cc of *Asellus* per female once or twice a week. When feeding was stopped, the average number of eggs laid sharply decreased and was statistically different from that of the preceding period.

number of eggs laid and the percentage of egg-laying females also varies. Table 4 shows that, in the course of several months, actual egg-laying varied significantly from the expected probability of random egg-laying. Thus, the hypothesis of random egg-laying is refuted. In the 28 month observation period, the average maximum temperature did not drop below 18°C, the minimum temperature did not drop below 15°C and egg-laying never stopped.

Further observations were made on specimens reared in a room facing north and given 2 cc of *Asellus* per female per day, in order to ascertain how the frequency of oviposition and the total number of eggs laid varied with seasonal variations in both temperature and photoperiod. A sample of 18 female *Nepa trinacriae* were kept under observation for 12 months. The correlation

TABLE 4 - *Variations in the number of mature females that laid eggs over a 28 month period between August 1978 and January 1981. Statistical analysis shows that variability was not random.*

Years	Months	Number of females		TOTAL
		non egg-laying	egg-laying	
1978	Sep	7	8	15
	Oct	8	8	16
	Nov	7	11	18
	Dec	4	15	19
1979	Jan	5	20	25
	Feb	2	28	30
	Mar	1	32	33
	Apr	11	21	35
	May	7	29	36
	June	4	32	36
	July	5	30	35
	Aug	7	30	37
	Sep	23	15	38
	Oct	18	19	37
	Nov	26	10	36
	Dec	10	27	37
1980	Jan	13	28	41
	Feb	2	41	43
	Mar	2	39	41
	Apr	7	34	41
	May	13	28	41
	June	11	26	37
	July	6	27	33
	Aug	7	24	31
	Sep	2	30	32
	Oct	8	30	38
	Nov	7	31	38
	Dec	21	14	35
TOTAL		244	690	934

$$n_{i.} = \sum_{j=1}^{2} n_{ij} \; ; \; n_{.j} = \sum_{i=1}^{28} n_{ij} \; ;$$

$$\sum_{i=1}^{28} n_{i.} = \sum_{j=1}^{2} n_{.j} = \sum_{i=1}^{28} \sum_{j=1}^{2} = n \; ;$$

$$\chi^2_{obs} = \frac{n^2}{n_{.1}\, n_{.2}} \left[\sum_{i=1}^{28} \frac{n_{i1}^2}{n_{i.}} - \frac{n_{.1}^2}{n} \right] ;$$

$$\chi^2_{obs} = \frac{(934)^2(94.724 - 63.743)}{(244)(690)} \; ;$$

$$\chi^2_{obs} = 160.5 \; ; \; \chi^2_{0.999} = 55.5 \; ;$$

$n_{i.}$, $n_{.j}$: marginal frequences ;

$n_{.1}$: total non egg-laying ♀♀ ;

$n_{.2}$: total egg-laying ♀♀ ;

between temperature and the percentage of egg-laying females ($r = 0.66$), as well as the change in mean egg-production per month with temperature and photoperiod, are shown in Fig. 10. It should be noted that, as the length of day-light and temperature decreased, there was a gradual reduction in the percentage of egg-laying females and a gradual decrease in the number of eggs laid per month. When the mean maximum and minimum temperature fell below 15° and 11°C, respectively, egg-laying stopped and the amount of food ingested by females was reduced to a minimum. However, when the length of day-light and the average temperature began to increase, the number of egg-laying females and the number of eggs laid sharply increased.

Eggs can be retained in the ovaries for variable periods of time. Indeed, up to some forty ovulated eggs can be stored inside the 10 pedicels of *Nepa trinacriae* and all of these can be laid at the same time, if conditions are suitable. The variability in the intervals between times of oviposition seems to be mainly related to the variations in the intervals between feeding. Indeed, in suitable environments, oviposition becomes more frequent, even daily, when females are supplied with food on a fixed schedule. Figure

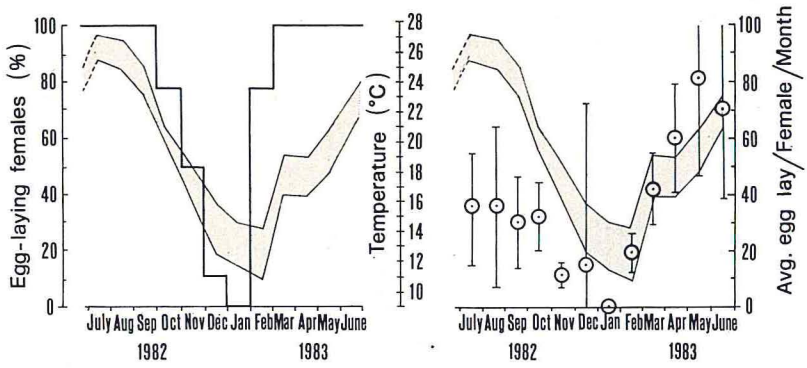


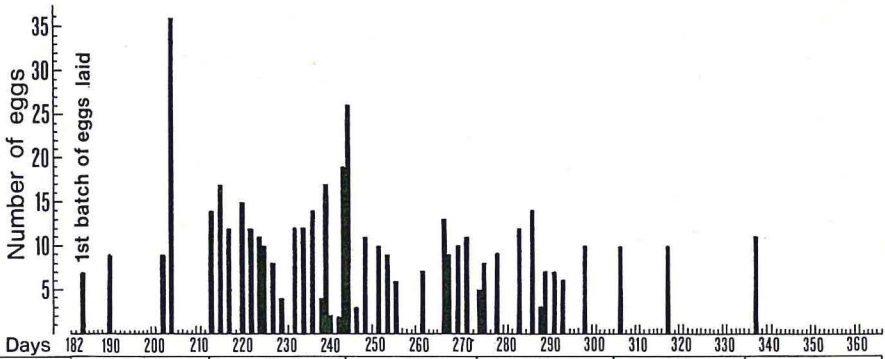
Fig. 10. Left - Correlation between the percentage of egg-laying females and the temperature during a 12 month observation period (r = 0.66). Dark area = average maximum and minimum temperatures.
Right - Monthly average of egg production. Data for each month include: average maximum and minimum temperatures (dark area); the average number of eggs laid per egg-laying female (dotted circles), the 95% confidence limits and corresponding confidence intervals. Eighteen female *Nepa trinacriae* were utilized for these observations, for a period of 12 months. They were reared in a room facing north and were fed 2 cc of *Asellus* per day.

11 shows the number of eggs laid per day during the first 365 days of reproductive activity of a female *Nepa trinacriae* that was fed 2 cc of *Asellus* per day. In only 12 months 1014 eggs were laid, a much larger amount than the maximum egg production by other females fed only once or twice a week.

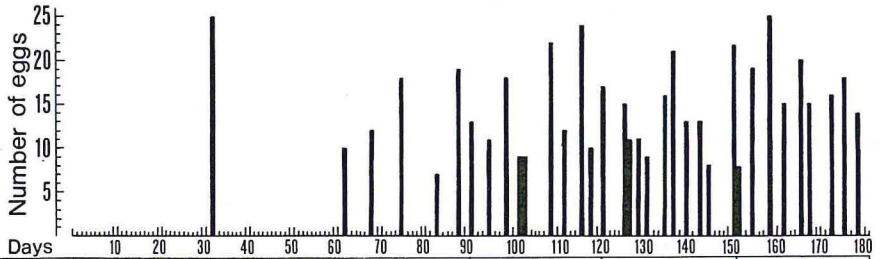
The experiment has been replicated several times with different females and the egg-laying pattern was the same.

The Duration of the Pre-Oviposition Period

Females are not mature at eclosion and their ovaries contain eggs in the previtellogenetic stage. A period of maturation is needed before an adult possesses eggs that are ready to ovulate. The delay between emergence and the beginning of vitellogenesis varies according to environmental conditions. The interval between eclosion and the first oviposition was recorded in 89 female *Nepa trinacriae* that emerged in different months of the year. Table 5 shows data obtained during three different 4 month periods. The average intervals in the first two periods are 80 and 82 days, re-



N	4	17	11	10	2	1
M± S	15.25± 13.87	10.68± 5.27	10.55± 5.85	8.10± 3.28	10± 0	
Σx	61	185	116	81	20	11
1982	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER



N	0	1	6	9	10	9
M± S			13.17 ± 4.62	14.67 ± 5.74	13.90± 4.70	16.67 ± 4.69
Σx	0	25	79	132	139	150
1983	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE

Fig. 11 - Bar diagram showing daily oviposition of female *Nepa trinacriae*, given 2 cc of *Asellus* per day, over the first 365 days of reproductive activity. Data for each month include: number of batches (N); number of eggs (ΣX); average number of eggs per batch (M); standard deviation (S).

spectively, and are not significantly different. The last period of the year, when the temperature and the length of day-light are decreasing, has a much longer preoviposition interval (115 days) which is statistically different from the intervals of the first period (Table 6).

The Developmental Period of Eggs

The length of the embryonic development of *Nepa trinacriae* is temperature dependent. In the laboratory, the upper threshold

TABLE 5 - *Average intervals (days) between eclosion and the first oviposition, recorded in 89 female* Nepa trinacriae *that emerged in different 4 month periods.*
A, B, C = first, second and third 4 month period; N = number of females; M = means; S = standard deviation.

4 Month periods	A Jan.Feb. Mar.Apr.	B May.June July Aug.	C Sept.Oct. Nov.Dec.	Total
N	12	24	53	89
M	79.583	81.875	114.87	101.21
S	31.592	51.984	57.909	55.594

TABLE 6 - *Analysis of variance for the data in table 5.*
D.F. = degrees of freedom.

Source of variation	D.F.	Sum of square	Mean square	f ratio
Comparison between 4 month periods				
(A+B) → C	1	24429.31	24429.31	8.49
A → B	1	42.01	42.01	< 1
Error	86	247513.63	2878.07	
Total	88	271984.95		

for the hatching of eggs lies between 36° and 42°C. The lower temperature threshold lies between 6° and 12°C; however, a small percentage of eggs, initially kept at 5°C for 50 days and then kept at room temperature, continued their development and hatched. Several coeval eggs were incubated together at a constant temperature and the day of incubation in which the first egg hatched was recorded. The number of days to first hatch was 7 at 32°C and 48 at 12°C.

The relationship between temperature and the rate of embryonic development may be rendered by an equation of type

$$\frac{100}{y} = \frac{K}{1 + e^{a-bx}}$$

where y is the time required for complete development, x is the temperature and K, a and b are constants (DAVIDSON, 1944). Days to first hatch and percentage development per day were both plotted against temperature, and a hyperbola and sigmoid curve, respectively, were obtained for *Nepa trinacriae* (Fig. 12).

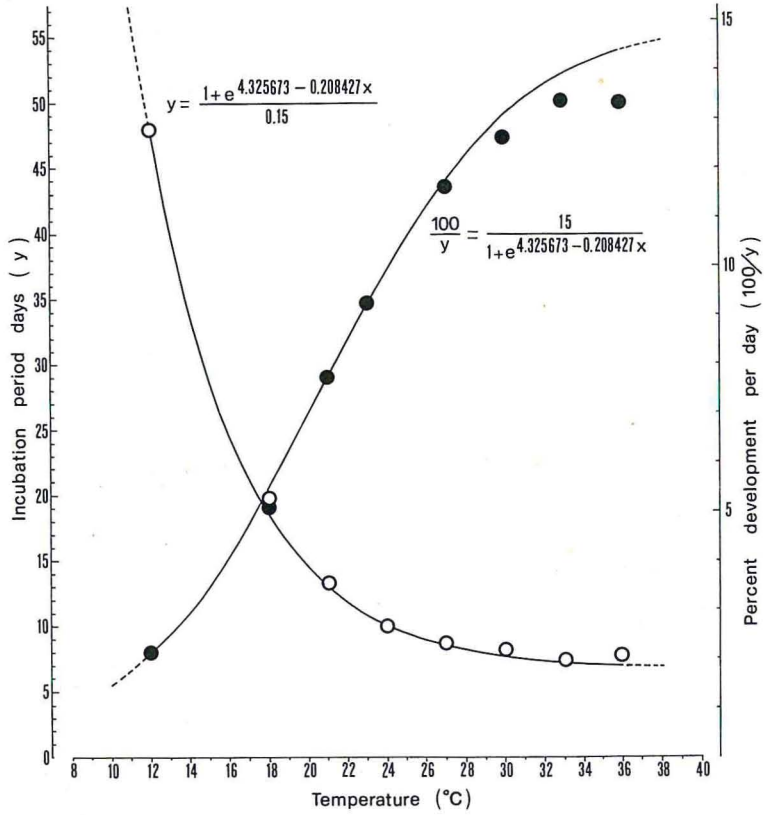


Fig. 12 - Relationship between temperature and development of eggs of *Nepa trinacriae* calculated by using day to first hatch since oviposition.

Eggs can also hatch even if they are completely submerged under water and newly hatched larvae are able to reach the surface of the water and complete their post-embryonic development. *Nepa trinacriae* eggs that were laid overnight by 5 female were submerged in 1 cm of water that did not contain any green filamentous algae. These eggs hatched normally when incubated at

23°C and the number of days to first hatch increased as the interval between egg-laying and submersion under water decreased.

The Katatrepsis of the Respiratory Horns

At the beginning of embryonic development, the respiratory horns are joined together and form a single bundle that sticks out of the water and above floating vegetation where the egg is situated. With time the respiratory horns spread apart i.e., they under-

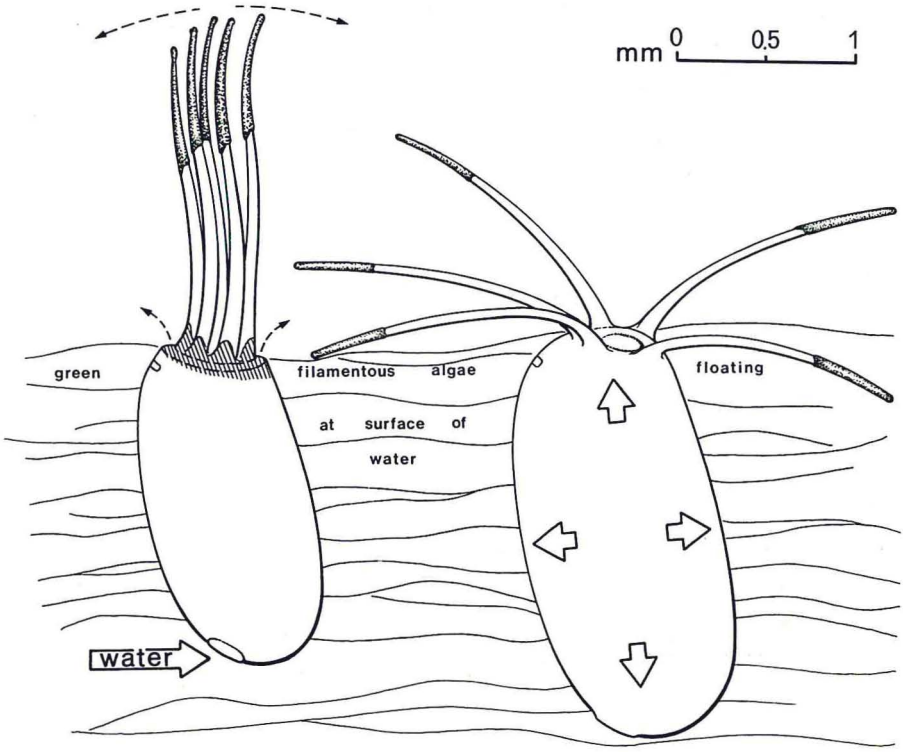


Fig. 13. Left - The katatrepsis of the respiratory horns. The respiratory horns, of a newly laid egg, stick out above floating vegetation. The air penetrates their pierced regions. A circular crown-shaped ring of air may collect around the opercular region.
Right - In advanced stages of embryonic development, the egg is greatly enlarged because of water that it has absorbed and because of change in the shape of its anterior pole and because of the elasticity of the egg shell. The crown-shaped ring of air disappears and the respiratory horns undergo katatrepsis remaining submerged under water until hatching.

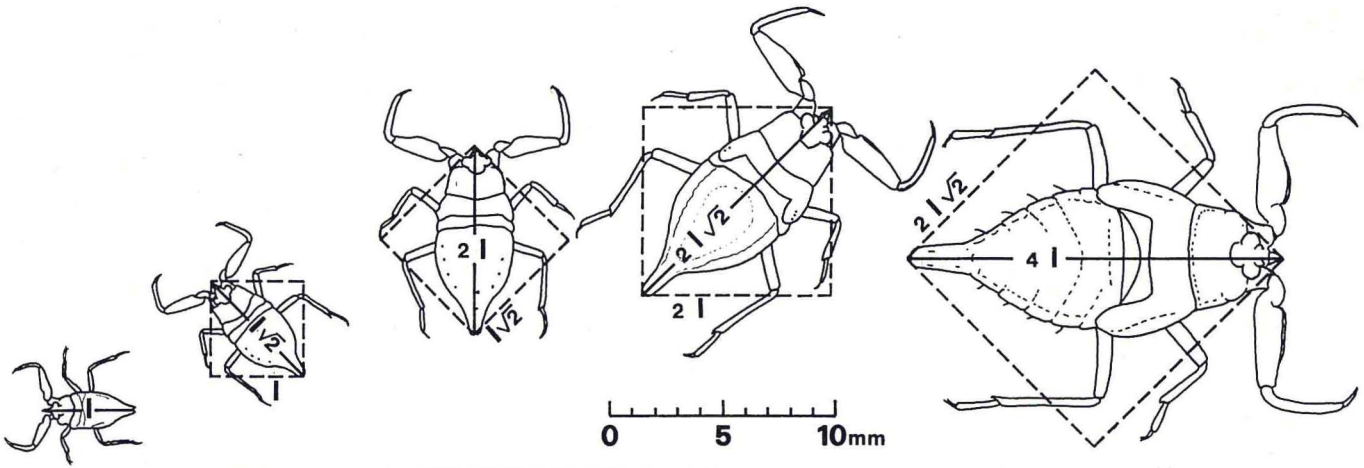
go a katatrepsis of 90° and come to lie on the algae on the surface of the water and are covered by a film of water (Fig. 13). At the end of embryonic development, the egg is greatly enlarged because of water that it has absorbed through the hydropyle. Both egg-size enlargement and the katatrepsis of the respiratory horns are possible because of the elasticity of the egg shell and because the opercular plate, under the pressure of the increasing water content of the egg, is first lifted up and then bulges out like a dome.

During embryonic development, the respiratory horns function in two different ways. At first, when they rise out of the water and above the algae, air penetrates the pierced regions and accumulates at the base of the respiratory horns. A circular, crown-shaped ring of air can collect around the opercular region. Later, in far-advanced stages of embryonic development, the above crown-shaped ring of air disappears and the respiratory horns undergo katatrepsis, remaining submerged underwater until the larvae hatch.

Eggs that have been laid at the same time usually hatch at the same time, although a small percentage of eggs hatch with a 24 or, at most, a 48 hour delay.

Larval Growth

During their post-embryonic growth period until they become adults, newly hatched *Nepa* larvae pass through 5 stages and moult their cuticle 5 times in order to increase their size. The study of the larval growth of the species of Italian *Nepa* provides additional information that refutes the validity of the general rules of growth in Arthropoda. The rate of growth in male *Nepa cinerea* is less than in females (MAZZA, 1975) and a similar situation exists in the case of *Nepa sardiniensis* and *Nepa trinacriae*. There are no statistical differences in the size of the newly hatched larvae of male and female *Nepa trinacriae*. The surface area of their flattened body approximately doubles with every moult (Fig. 14). The hypothetical progression factor of linear growth inferred from this observation is $\sqrt{2}$. This factor is true for females (Table 7. Left) but not for males (Table 7. Right). For a sample of 13 male *Nepa trinacriae*, reared in the laboratory, the progression factor of linear growth was 1.384 ± 0.04371.



	1st INSTAR		2nd INSTAR		3rd INSTAR		4th INSTAR		5th INSTAR	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
N	14	13	20	14	19	13	20	13	20	13
M	3.9524	3.9679	5.3792	5.5238	7.5395	8	10.492	11.231	14.525	16.013
S	0.18979	0.16506	0.22373	0.26032	0.29050	0.31180	0.41789	0.33705	0.53674	0.64983
	$F = 0.052$; $F_{0.05} = 4.2$		$F = 3.0$; $F_{0.05} = 4.2$		$F = 18$; $F_{0.05} = 4.2$		$F = 28$; $F_{0.05} = 4.2$		$F = 51$; $F_{0.05} = 4.2$	

Fig. 14 - Larval growth of *Nepa trinacriae*. Sketches of the complete series of cast skins of a female *Nepa trinacriae*; the progression factor of the length proves to be $\sqrt{2}$. The data contained in the tables clearly show there are ever-increasing differences between the average lengths of the males and those of the females in the series of larval stages. The measurements are given in millimetres. The lengths of the specimens were measured from the tip of the rostrum to the tip of the abdomen. N = number of specimens measured; M = average length; S = standard deviation; F = F test.

TABLE 7 - *Length, measured in W.U.6, of the complete series of cast skins shed by 13 female* Nepa trinacriae. *The hypothetical progression factor of linear growth, $\sqrt{2}$, fits the data well.*

N = number of specimens measured; M = mean length observed; μ = theoretical mean length assessed with $\sqrt{2}$, the hypothetical progression factor of linear growth; $M \pm t_{0.05} S_m$ = confidence limits.

FEMALES	1st INSTAR	2nd INSTAR	3rd INSTAR	4th INSTAR	5th INSTAR
LARVAL LENGTH IN W.U.6	24	35	50	70	101
	26	34	50	70	99
	25	35	50	69	95
	25	35.5	51	70	100.5
	23	32.5	45.5	68	96
	23	32	47	67	100
	24	34.5	47	66	95
	23.5	33	47.5	68	97
	23	32.5	48	65	92
	23	32.5	46.5	64	86.5
	24	33	47.5	67	97
	23	31	45	65	95
	23	34	49	67	95
N	13	13	13	13	13
M	23.808	33.423	48	67.385	96.077
S	0.99034	1.3516	1.8708	2.0223	3.899
$M+t_{0.05}$ Sm	24.406	34.240	49.131	68.607	98.434
μ	23.808	33.669	47.615	67.338	95.231
$M-t_{0.05}$ Sm	23.209	32.606	46.869	66.162	93 720

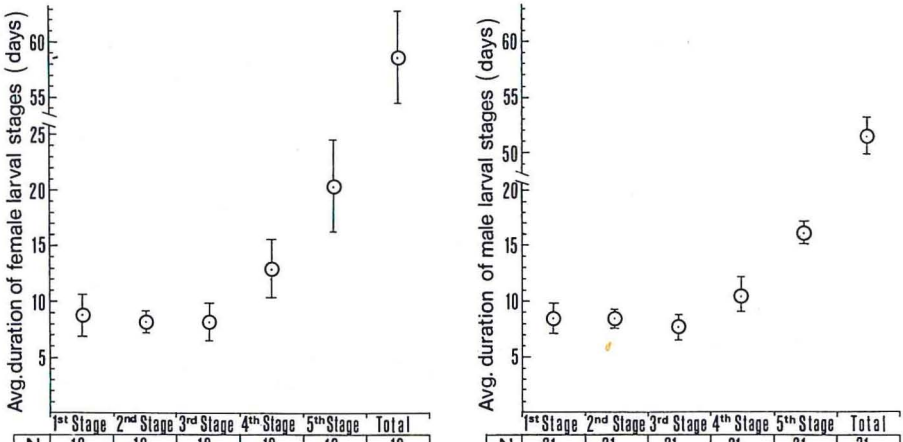
TABLE 8 - *Length, measured in W.U.6, of the complete series of cast skins shed by 13 male* Nepa trinacriae. *The hypothetical progression factor of linear growth, $\sqrt{2}$ does not fit the data. For the length of cast skins at every single stage as divided by the preceding one, the quotient found is 1.384 ± 0.04371.*

N = number of specimens measured; M = mean length observed; μ = theoretical mean length assessed with $\sqrt{2}$, the hypothetical progression factor of linear growth; $M \pm t_{0.05} S_m$ = confidence limits.

MALES	1st INSTAR	2nd INSTAR	3rd INSTAR	4th INSTAR	5th INSTAR
LARVAL LENGTH IN W.U.6	24.5	33	47	66	88
	26.5	34	49	66	90
	24.5	34	46	61	88
	25	34.5	46	66	88
	24	32	45	63.5	88
	24	33	45	63.5	88.5
	23.5	32	44	61	87.5
	23	32.5	47	65	92
	23	31.5	43.5	63.5	89
	23	29.5	44.5	60	86
	23	33	46	64	88
	23	31	43	59	81.5
	23	30	42	58.5	80.5
N	13	13	13	13	13
M	23.846	32.308	45.231	62.846	87.308
S	1.0682	1.5212	1.887	2.6723	3.1327
$M+t_{0.05}$ Sm	24.492	33.227	46.373	64.462	89.202
μ	23.846	33.724	47.692	67.447	95.385
$M-t_{0.05}$ Sm	23.200	31.388	44.089	61.230	85.414

The Duration of the Larval Stages

In general, the duration of the larval stages is food and temperature dependent. *Nepa trinacriae* larvae did not moult when the mean minimum daily temperature was below 11°C and when the mean maximum daily temperature had not risen above 14°C. The duration of the individual larval stages was measured in the laboratory during July and August 1982 when the mean minimum and maximum temperatures were 23°C and 26°C respectively. The duration of each larval stage was not the same. The first three stages were the shortest and there was no statistical difference between them. The fourth and fifth larval stages were the longest and the fifth was statistically longer than the fourth.



	1st Stage	2nd Stage	3rd Stage	4th Stage	5th Stage	Total
N	16	16	16	16	16	16
M	8.812	8.250	8.187	12.937	20.375	58.562
S	3.468	1.844	3.060	5.013	7.684	7.840
Σx	141	132	131	207	326	937
Σx^2	1423	1140	1213	3055	7528	55795

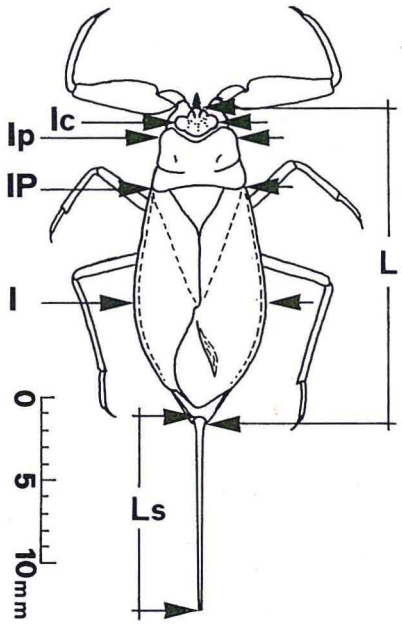
	1st Stage	2nd Stage	3rd Stage	4th Stage	5th Stage	Total
N	21	21	21	21	21	21
M	8.524	8.524	7.714	10.619	16.143	51.524
S	3.027	1.692	2.648	3.708	2.242	3.696
Σx	179	179	162	223	339	1082
Σx^2	1709	1583	1390	2643	5573	56022

Fig. 15 - Mean duration of each larval stage of 16 female (left) and 21 male (right) *Nepa trinacriae* reared in the same laboratory conditions. The graph shows the mean (dotted circles) and 95% confidence intervals.

The mean duration of each larval stage studied, the 95% confidence limits and other statistical parameters are shown in Fig. 15.

The Size of Adults

The body of adult *Nepa trinacriae* is very broad and flat, with a respiratory syphon that protrudes from the apex of the abdomen. The head is small and the eyes are small and globular. The short antennae are hidden in a groove below the eyes. The rostrum has three segments. The prothorax is wider than the head and its anterior margin has a semicircular notch where the head lies. The forelegs are raptorial and the femur is slightly longer than the tibia. The morphology of the males and females is similar but the former are smaller than the latter. Figure 16 con-



	N	M	S
L ♀♀	46	18.707	1.1543
L ♂♂	43	15.864	0.72398
Ls ♀♀	46	10.848	0.92618
Ls ♂♂	43	9.0000	0.69007
Ic ♀♀	46	2.3078	0.076185
Ic ♂♂	43	2.0381	0.069941
Ip ♀♀	46	4.2645	0.23459
Ip ♂♂	43	3.8023	0.13242
IP ♀♀	46	5.6051	0.33214
IP ♂♂	43	4.8876	0.23774
I ♀♀	46	7.4746	0.44301
I ♂♂	43	6.1512	0.28128
I/Ic ♀♀	46	0.38836	0.014022
I/Ic ♂♂	43	0.36225	0.012539

Fig. 16 - Table containing data and a sketch of a female *Nepa trinacriae* with a diagram of the points where measurements were taken. The measurements are in millimetres. The last lines in the tables contain the average values of the relationship between the width of the abdomen, measured in w.u. 6, and the width of the head, measured at the eyes, in w.u. 50. The value of this quotient is sufficient to distinguish *Nepa trinacriae* from *Nepa cinerea* but not from *Nepa sardiniensis*.

tains a data table and a sketch of a female with a diagram of where measurements were taken. The value of the quotient between the width of the abdomen and that of the head is sufficient to distinguish *Nepa sardiniensis* from *Nepa cinerea* and *Nepa cinerea* from *Nepa trinacriae.* Certain environmental conditions such as starvation during larval stages can cause up to a 20% reduction in the mean size of both males and females.

The Morphology of the Antennae

The antennae of *Nepa* have only three segments. Their morphology was first used by HUNGERFORD (1922) to classify many species of water scorpions and, thereafter, was considered to be extremely important for the taxonomy of Nepidae. For the identification of Italian species, however, they are of limited value because the morphology of the antennae of *Nepa cinerea* varies greatly (MAZZA, 1975), and the morphology of the antennae of *Nepa trinacriae* and *Nepa sardiniensis* are very similar. The antennae of *Nepa trinacriae* is the same shape in both sexes. A small apophysis with quite variable size and shape is located in the second segment. The drawings in figure 17 show the variability of the morphology of the antennae of male and female *Nepa trinacriae.*

CONCLUSIONS

The Nepidae of Italy known at present are *Nepa cinerea, Nepa sardiniensis* and *Nepa trinacriae.* If we wish to distinguish between *Nepa trinacriae* and other Italian water scorpions, the morphological characteristics traditionally used by authors are of no avail because the size of the body and the morphology of the antennae of *Nepa cinerea* are variable to an extent which includes *Nepa sardiniensis* and *Nepa trinacriae.*

These two latter species are very similar to each other. Moreover, the relationship between the width of the abdomen and that of the head, which is sufficient to recognize *Nepa cinerea* (MAZZA, 1976), is not statistically different in the case of *Nepa trinacriae* and *Nepa sardiniensis.*

Certain aspects of the reproductive biology of *Nepa trinacriae*

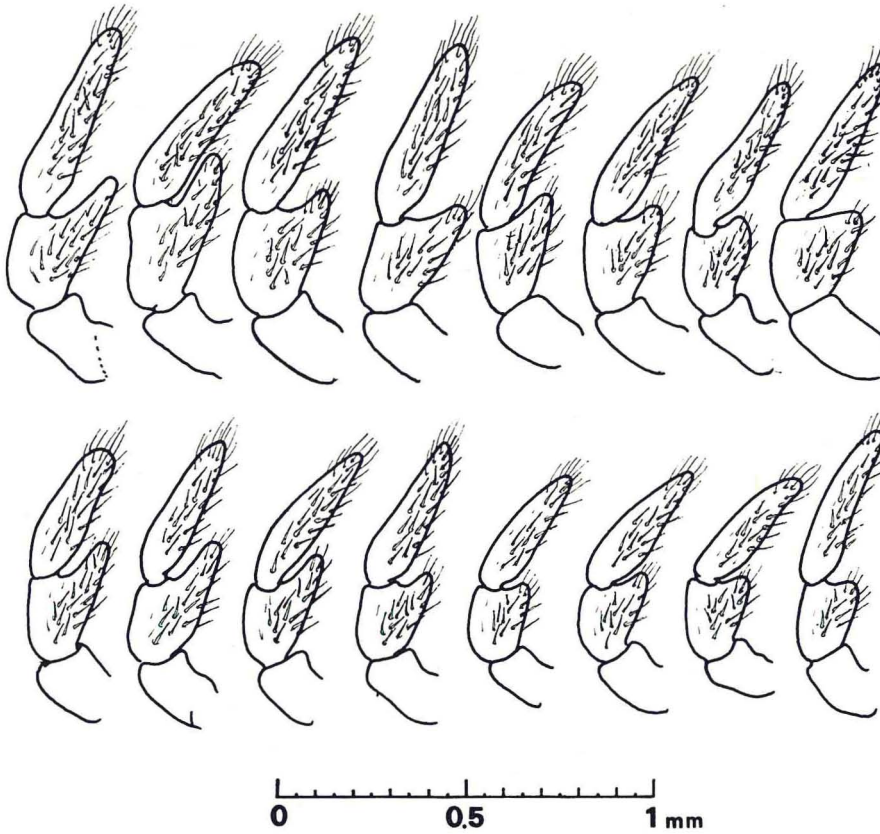


Fig. 17 - Antennae of female (upper strip), and male *Nepa trinacriae*.

make it possible to characterize this species better. The eggs are equipped with long appendages used for breathing, called respiratory horns and produced by large-size follicular cells.

The majority (66.5%) of the eggs examined (25154) have 6 respiratory horns but the number is never constant and, under the influence of external environmental factors, they may vary from 3 to 11. Only 29.3% of the large sample of eggs examined have 5 respiratory horns, but eggs of this kind may represent the most common type laid by a given female. The fact that females reared in the same laboratory conditions produce a majority of eggs with 5 or 6 respiratory horns does not seem to be a random event but, rather, seems to be associated with factors transmitted from generation to generation.

The number of respiratory horns on the eggs has been used to classify the Nepidae (HINTON, 1962). But such a criterion is of little use for the identification of the Italian species because the number of respiratory horns of the eggs of *Nepa cinerea* is variable to an extent which includes *Nepa sardiniensis* and *Nepa trinacriae,* and because the variations in the number of respiratory horns on the eggs of *Nepa sardiniensis* and on those of *Nepa trinacriae* are similar. Whereas the respiratory horns are much more useful for the purposes of classification if they are measured. The measurement of their length alone is enough to be able to distinguish between the various species of Italian *Nepa.*

The number of eggs that can both mature and be laid varies from one individual female to another and also depends upon temperature, the photoperiod and, above all, food. All conditions being equal, *Nepa trinacriae* is able to lay definitely more eggs than *Nepa sardiniensis* and for this reason the data reported here are useful for distinguishing between these two species.

As in the case of other insects (WIGGLESWORTH, 1965; HOWE, 1967; KAY, 1981), the development time of the eggs of *Nepa trinacriae* is directly related to temperature.

The start of hatch, of coeval eggs, ranges from first hatch on day 7 at 32°C to first hatch on day 48 at 12°C. A similar relationship was found in the case of the burrowing mayfly *Hexagenia rigida* (FRIESEN et al., 1979).

The relationship between temperature and development time is complex; nevertheless, a number of mathematical equations have been worked out to describe it (DAVIDSON, 1944; PRADHAN, 1946; WIGGLESWORTH, 1965). The equation for *Nepa trinacriae* is

$$y = \frac{1 + e^{4.325673 - 0.208427\,x}}{0.150}$$

where y is the time, in days, and x is the temperature (°C).

The other data available at present do not allow us to compare the various hatching times of the Italian *Nepa.*

During the embryonic development of *Nepa trinacriae,* the egg undergoes clear changes since it increases in volume by adsorbing water, and because the respiratory horns carry out a katatrepsis of 90°. Both enlargement in egg-size and katatrepsis of the respiratory horns are possible because of the elasticity of the chorion

and because the opercular plate, under pressure from the increasing water content of the egg, is, first, lifted up and then bulges out like a dome.

At an advanced stage of embryonic development, the respiratory horns open out, like daisy petals, making way, among the green filamentous algae, for the larva which will emerge from the egg a few days later.

When the katatrepsis is completely finished, the respiratory horns remain under a film of water until the egg hatches, and throughout this entire period O_2 alone, dissolved in water, is used.

Newly-hatched larvae pass through 5 stages and moult their cuticle 5 times in order to increase their size. Certain empirical laws have been formulated to describe larval growth in Arthropoda. BROOKS (1886) seems to have been the first to try to predict the amount of growth from one stage to another. He used this method to determine the number of larval stages of certain species of Stomatopoda for which the complete series of larval stages had not yet been established. DYAR (1890) stated that the width of the head and other parts of the body of Lepidopterous larvae increases by a geometrical progression each moult and has a growth coefficient that is constant for a particular species. In 1949, Richards showed that Dyar's rule holds good only when each larval stage has the same duration, otherwise the change in size is proportional to the time which an instar lasts.

PRZIBRAM (1912) studied the growth of *Sphodromantis* larvae and found that larval weight doubled at every stage. He stated that the number of cells in each successive larval stage doubled and therefore that the coefficient of linear growth should always be $\sqrt[3]{2}$. Przibram found the same coefficient of linear progression on the basis of measurements made by BROOKS (1886) in certain Stomatopoda and considered his coefficient to be valid for the growth of all Arthropoda. BODENHEIMER (1927, 1932) carried out similar studies on many orders of insects and found numerous instances in which larval weight increased two or more times between successive moults. He stated that non-manifested ecdysis or «latent divisions» obscure Przibram's rule without invalidating it. Przibram's rule has been subsequently invalidated for other reasons, one of the most important being that the rule assumes that insect growth is isogonic whereas it is generally heterogonic.

Other important reasons are that, in certain Diptera, the cells increase in size in successive larval stages without dividing, and, at least in *Rhodnius,* it is the increase in area of the tegument which determines the increase in the number of epidermal cells and not the contrary (WIGGLESWORTH, 1937; CHAPMAN, 1978; GILLOT, 1980).

The study of the larval growth of the species of Italian *Nepa* provides additional information with which to refute the validity of the general rules of growth in Arthropoda. In female *Nepa cinerea, Nepa sardiniensis,* and *Nepa trinacriae,* the progression factor of linear growth is $\sqrt{2}$. But the rate of growth in males is less than in females. For a sample of male *Nepa trinacriae* reared in the laboratory, the progression factor of linear growth was 1.384 ± 0.04371.

Longevity of adult water scorpion ranges from 1 to 3 years. During the greater part of this time, males and females may be able to reproduce but at eclosion they are not yet sexually mature. Males can copulate but eggs are not fertilized. If conditions are suitable, female *Nepa trinacriae* may be ready to ovulate one month after emergence, the eggs may hatch 8 days after oviposition and and newly hatched larvae may become adult in 45 days. It is well established that *Nepa trinacriae* is polivoltine.

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