Life History Studies of the Flightless Marine Midges *Pontomyia* spp. (Diptera: Chironomidae)

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Keryea Soong, Guo-Fang Chen and Jun-Ren Cao (1999) Life history studies of the flightless marine midges *Pontomyia* spp. (Diptera: Chironomidae). *Zoological Studies* 38(4): 466-473. The emergence of the marine midges, *Pontomyia natans* and *P. oceana*, was investigated in southern Taiwan in 1992 and 1996. *P. oceana* emerged after sunset and only on evenings near the new moon or the full moon, whereas *P. natans* emerged near dusk on every collection day. The mean emergence time of *P. oceana* varied by more than 3 h, being earlier in summer than in winter. The overlap in emergence times of the 2 species thus depended upon the season. Light conditions, i.e., the time of sunrise and sunset may be the proximate factors controlling emergence in *P. oceana*. Limiting emerging dates and times of *P. oceana* may be an adaptation for controlling the adults to lay eggs only during low tides at night. The tide, however, does not cue the diurnal emergence of the midges. Fertilized eggs of *P. oceana* were collected in the field for study in the laboratory. Larvae hatched in about 4 d at 25 °C, and 4 instars were necessary before the final emergence. The generation time was around 30 d. Males emerged about 1 h earlier than females. Since an adult remained active for only about 2 h, peak occurrence of males was found to coincide with peak eclosion of females. Moreover, the presence of males was important for the successful eclosion of females from their pupae, as demonstrated in the laboratory.

**Key words:** Marine insects, Eclosion, Intertidal.

The adult stage of many organisms is often the longest compared with other stages in the life history. It is also the stage in which most species are recognized, and at which their biology is best understood. There are, however, some species whose adult stage is relatively short, with its main role limited to reproduction (Neumann 1976). Most adaptations in adults of these species are thus aimed directly at reproductive success rather than for growth and survival. The life histories of marine midges in the genera *Pontomyia* and *Clunio* (Chironomidae), for example, consist of a relatively long period (≥ 1 mo) of benthic larval development, followed by a very short (1-2 h) adult stage. In these sexually dimorphic species, males must find mates and females, besides mating, must deposit fertilized eggs in appropriate habitats during their short lives (e.g., Neumann 1976, Cheng and Hashimoto 1978).

Time of emergence is critical for these short-lived species, and it has been the focus of many studies. Midges inhabiting intertidal areas often emerge following a semi-lunar pattern, suggesting that tidal level might be an important ultimate controlling factor (e.g., Neumann 1966 1986, Saigusa and Akiyama 1995). Egg strings of these species are often laid such that they adhere to hard substrates. Thus, the need for exposure of a substrate for egg-laying and the later immersion of the eggs would select for adults which emerge some time near low tide. For the same reason, they would prefer spring tides when more time is available before the tide rises again. Both a semi-lunar and a diurnal pattern of emergence, therefore, might be adaptations to tidal fluctuations in their habitats. Species inhabiting sub-littoral areas, in contrast, do not require such adaptations. They usually have sinking eggs (Endrass 1976), and the emergence of adults is often not limited to days of spring tides (e.g., Heimbach 1978, Neumann 1986). On emergence days, the short duration of adult emergence may also be selected for

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by factors other than tidal fluctuations (Neumann 1976). The environmental cues entraining this periodicity may differ among species or may depend on the environment of the local population (Neumann 1980). It has also been noted that males often emerge earlier than females (Hashimoto 1962, Neumann 1966, Heimbach 1978, Cheng and Collins 1980). The possible adaptive value and mechanism of this sexual difference in timing remains to be investigated.

Despite numerous studies on marine midges, especially in the genus Clunio (e.g., Neumann 1988), little is known about Pontomyia spp. that are distributed in the western Pacific (Cheng and Hashimoto 1978). In this study, life history characteristics of Pontomyia natans Edwards, 1926 and P. oceana Tokunaga, 1964 were investigated in the field and in laboratory cultures.

**MATERIALS AND METHODS**

**In the field: time of emergence**

A standard sampling technique was employed to collect live midges in intertidal zones after dark. A 6-W flashlight was held 15-20 cm above the water surface for 30 s to attract the highly mobile male midges skiing on the water surface. Then, a previously submerged square net (50 x 50 cm) was pulled up to collect the midges. Only males with high mobility were attracted to the light. The degenerate, vermiform females (see Cheng and Hashimoto 1978) were caught when they were copulating with, and being dragged by, males. The midges could be efficiently gathered from the net with a pair of forceps or a fine brush rinsed with alcohol. Collected midges were preserved in 80% ethyl alcohol.

Lunar variation in the emergence of marine midges was investigated at 2 sites, Wanliton (22°00′N, 120°42′E) and Howan (22°03′N, 120°41.5′E), 5 km apart from each other in southern Taiwan. Samples were collected every 3rd night between 15 July and 15 August 1992. The collection sites were located in the high littoral zone which consists of a series of tidal pools at low tide. On each night of collection, 2 samples were collected every 30 min starting at 18:00 hours until no further midges appeared.

An additional 20 nights of samplings were conducted, mostly at Wanliton, between 21 August and 24 December 1992 and between 5 March and 2 April 1996. Mean water temperature was obtained by averaging water temperatures measured during each collection. The times of sunrise and sunset were taken from a local astronomer’s almanac (Anonymous 1993). The times of high and low tides were obtained from local tide tables (Anonymous 1992 1996). The lunar day is designated as in the lunar calendar, with day 1 indicating a new moon, day 15 indicating a full moon, etc. These data were used to analyze correlations between emergence and prevailing environmental factors. After returning to the laboratory, adult midges were sorted by species (using the key in Cheng and Hashimoto 1978), and numbers of males and females in each collection were counted. Mean emergence time was calculated by averaging the time of catch weighted by the number of individuals caught. To investigate possible day-time emergence, a 54-h collection at 2-h intervals was made on 5-7 December 1992. In addition, a hand net was used to sweep the water surface at 0.5-h intervals both during the day and night on 19 and 20 May 1997, before low tides.

The size structure of larvae was investigated at Wanliton on 30 May 1996, two days before the full moon. Larvae were shaken off dead coral fragments and rubble collected from intertidal pools where the adults were captured. The head capsule widths of these larvae were measured in the laboratory using a calibrated stereo-microscope.

**In the laboratory: life cycle**

Live midges were attracted into a transparent bottle in the field by light and were brought back to the laboratory and put into containers. After females had deposited egg strings on the bottom of the containers, the containers were filled with sea water to keep the fertilized eggs immersed. Eggs hatched in 3-4 d, and the larvae were transferred to culture trays containing large pieces of dead coral fragments. A 12D/12L photoperiod with light from 06:00 to 18:00 hours was used as normal laboratory conditions. Four 30 W day-light fluorescence tubes were fixed about 30 cm from the water surface, at an average light intensity of 6500 lux. The water temperature of the culture trays was maintained at 25 °C. powdered fish meal or dried Ulva powder was added at 2-d intervals to feed the larvae. No tidal fluctuation was simulated in the laboratory. The total body length and head capsule width of a cohort of larvae were monitored by measuring about 20 larvae every other day.

Whereas “emergence” was defined as the appearance of live adults, it is preceded by “eclosion” of pupae during which they must shed their skin at the water surface. Emergence of midges in the laboratory occurred about 30 d after egg hatching. Pupae
and adults were collected from the water surface by a net once every 15 or 30 min, beginning at 19:00 hours on nights of emergence, under dim red light. Pupae were put in separate cups to record duration of active skiing for males, and time taken for females to deposit eggs. Except for some females, eclosion usually occurred within 1 min after collection. The nightly mean emergence times of the laboratory cultures were calculated in a way similar to that used in the field. The emergence time for all individuals caught during a period was pooled, e.g., those collected between 19:45 and 20:00 hours were assigned an eclosion time of 19:52.5. This calculated eclosion time was considered to be an unbiased estimate of the true eclosion time. In contrast, only a small fraction of the total adults emerging in the population was caught in the field. Thus emergence time in the field reflects density of active individuals. The true eclosion time of females are known only in the laboratory.

An experiment was designed to investigate whether presence of males might play a role in the eclosion of females in *P. oceana*. A total of 36 female pupae were collected and separated into 3 groups. Twelve live males were put in the 1st; 12 dead males collected the previous night were put in the 2nd; and the 3rd control group included no males. The number of females emerging in each group were recorded after 30 min.

**RESULTS**

**In the field: lunar pattern of emergence**

There were 11 collecting nights between 15 July and 15 August 1992. At Howan, *P. natans* was caught on all 11 collecting nights. The total number of midges collected each night varied between 139 and 2064 (lower panel of Fig. 1). Two peaks of emergence occurred on lunar days 26 (25 July) and 11 (9 August), with 15 d apart (Fig. 1). At Wanliton, only a few *P. natans* were collected (a total of 60 individuals for the entire study period), and the pattern of their emergence was not analyzed.

In contrast, *P. oceana* was caught only on 5 nights at Howan and 4 nights at Wanliton between 15 July and 15 August 1992 (lower panel of Fig. 1). Additional collections at Wanliton and Howan were used to analyze the lunar pattern of emergence. It was found that nights with a significant number of emerging adults fell within 7 d of the new moon (lunar days 26 to 2) or the full moon (lunar days 11–17) (upper panel of Fig. 1). Of more than 70 000 *P. oceana* collected, only 2 individuals were found in a total of 16 nights outside these periods.

**In the field: diurnal patterns of emergence**

A significant linear regression (female time = $-9.5 \text{ min} + 1.1 \times \text{male time}, R^2 = 0.98$) was found between the mean emergence times of males and females on different collecting days in *P. oceana*. The slope and the intercept are not significantly different from 1 and 0, respectively. Thus, all analyses of the field data were based on those of males. In *P. natans*, only males were caught for the entire study period.

At Howan, the mean time of emergence of male *P. natans* mostly occurred between 19:04 and 19:47 for all collections between 16 July and 22 August 1992. The earliest mean time of emergence in this investigation was at 18:33 on 8 September. The mean time of emergence of male *P. oceana* occurred between 19:40 and 20:46, about 0.5 - 1 hour later than that of *P. natans*. The peak time of the catch usually occurred within 15 min of the mean time. There were differences in the overlap of emergence times on days both species emerged. A complete overlap in the duration of emergence of the 2...
species was recorded at Howan on 25 and 28 July 1992 (Fig. 2). On the other hand, a low degree of overlap, or no overlap at all, was recorded in other (Fig. 2) and especially winter collections, when the emergence of *P. oceana* was much later than that in summer.

At Wanliton, data for mean emergence time of *P. oceana* were available from July to December 1992 and from March to April 1996. A difference of more than 3 h (19:41 and 23:01) was found among samples at different times of the year. It occurred much earlier in summer (all before 21:00, except 1, \( n = 11 \)) than in winter and spring (November and April, all after 21:00, \( n = 13 \)). Only 60 males of *P. natans* were caught in 3 collections at Wanliton between November and December 1992; their mean emergence times were between 18:36 and 18:48.

The mean time of emergence of both species was found to be significantly correlated to times of sunrise and sunset, and mean water temperature (Table 1). In neither species were the mean times of emergence correlated with times of the previous high or low tides. However, the emergence time of *P. oceana* was correlated with times of the subsequent low tide (Table 1). Intervals between the emergence times and times of low tide were found to increase by about 50 min/d, the expected delay of tides per day.

Day-time collections yielded no *P. oceana* either in December 1992 or in May 1997, when the spring low tides occurred in the evenings and in daytime, respectively.

Suitable habitats of *P. oceana* are obviously not continuous along the coast. Even at Wanliton, the distribution is patchy; some tidal pools have no midges, while others might have thousands. Several sites sampled between Wanliton and Howan produced no midges.

**In the field: mating ratio and larval size**

The relative frequency of males with a copulating female in the field, here defined as the mating ratio, varied greatly among collections. Only 6% of males had mates in the collections of 24 December, whereas 70%, the highest mating ratio, was recorded on 25 July 1992. With all the data combined, 30.0% of males collected in the field had females with them. On individual collecting nights, peak number of midge emergence were often associated with high mating ratio (Fig. 2).

The larvae of *P. oceana* collected from intertidal substrate 2 d before the full moon could be divided into 2 distinct size groups with peak head capsule widths of 0.10 mm and 0.19 mm, respectively. They presumably represent cohorts 15 d apart.

**In the laboratory: life cycles**

Pupae of *P. oceana* floated to the surface a few minutes before eclosion. Males emerged from the pupal case and stretched their appendages above the water surface. About 10 s after eclosion, they began skiing on the water surface presumably searching for females. Females remained motionless after eclosion, with their tail segments hanging onto the water surface. Considering their small size (about 3 mm in total length), males moved at great

<table>
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<tr>
<th>Table 1. Correlation coefficients of 6 environmental factors to the mean time of emergence of male midges of <em>Pontomyia</em> spp.</th>
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<tbody>
<tr>
<td><strong>Environmental factor</strong></td>
</tr>
<tr>
<td>Sunrise time</td>
</tr>
<tr>
<td>Sunset time</td>
</tr>
<tr>
<td>Previous high tide time</td>
</tr>
<tr>
<td>Previous low tide time</td>
</tr>
<tr>
<td>Subsequent low tide time</td>
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<td>Average temperature (°C)</td>
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*p < 0.01; *0.01 < p < 0.05; **p > 0.05.*
speed. They did not appear to slow down after catching females, sometimes climbing the vertical substrate up to 15 cm above the water level while dragging a female along. After copulation, males may look for another mate. In the laboratory, unmated males remained active (157 ± 10 [S.E.] min; n = 33), significantly longer (p < 0.01, Mann-Whitney test) than did mated males (81 ± 10 min, n = 7). No difference was found between longevity of mated (131 ± 15 min, n = 4) and virgin females (139 ± 8 min, n = 41).

After mating, each female laid a continuous sticky egg string which adhered to the surface of dead coral or other available substrate near or above the water level. Unmated females were also observed to lay egg strings in the laboratory. The number of eggs in each string was positively correlated to body length of females (Number of eggs = −19.2 + 56.3 × total length [mm], r = 0.61). Up to 200 eggs, each measuring 180 mm in length, may be laid by a single individual. After fertilization, germ bands formed in 2 h; segments could be clearly distinguished in 50 h; and hatching occurred in about 90 h under laboratory conditions. Newly hatched larvae remained free-living on or above the substratum for 1 to a few days. Afterwards, they settled and began to build nest-tubes on the surface or in crevices of dead coral skeleton or other substrate, using small pieces of debris and fine sand.

Sizes of larvae were measured in a batch culture in spring 1993. Four molts were observed between hatching and pupal formation (Fig. 4). The 1st instar lasted about 5 d and averaged 0.064 mm in head capsule width. The 2nd instar lasted about 10 d, and the head capsule width increased to about 0.10 mm. The 3rd instar first appeared on the 12th day and could still be found on the 32nd day with a head capsule width of about 0.13 mm. Only a few individuals of the 4th and presumably the last instar were found; they had a capsule width of about 0.18 mm. The body length of larvae increased from about 0.64 mm in the beginning to an average of 2.6 mm when 1st adult emergence was recorded, 22 d after eggs hatched. A linear relationship was found between body length and head capsule width of larvae (Body length = −0.32 mm + 19.4 × Head capsule width, r = 0.94, p < 0.01). The sex of individual larvae could not be identified except for red, developing eggs which could be seen through the body wall of final instar females.

In the laboratory, first adult emergence occurred 26 d after eggs were fertilized, and emergence continued for the next 10 consecutive nights. Thereafter, only sporadic emergence occurred. In other batches of larvae raised in 1996, emergence occurred as early as 17 d and as late as 51 d after egg fertilization. No emergence occurred during the light. Males emerged on average 61 min earlier than females (see Fig. 5). Sex-ratio of laboratory-raised midges (191: 297) was significantly different from 1: 1, being biased toward females (Chi-square test, p < 0.01).

In the experiment testing the effects of males on female eclosion, 10 out of 12 females emerged in the presence of live adult males within 30 min, while only 2 and 1 emerged in the presence of dead males, or without males (control), respectively (p < 0.01). Subsequently, when 10 live males were added to the treatment with dead males, all 10 remaining females emerged within 30 min. No live males were added to the control group and no additional females emerged.

**DISCUSSION**

Several differences were found in the emergence patterns between the 2 marine midge species in this investigation. The lunar pattern observed in *P. oceana* is less obvious in *P. natans* (Fig. 1). This might be related to the different larval habitats of the 2 species. Egg strings of *P. oceana* adhere to surfaces of the substratum above the tidal water level. Thus, timing of mating and egg-laying at low tide...
would be critical for the survival of fertilized eggs. *P. natans*, on the other hand, inhabits the sublittoral zone (Marks 1971, Cheng and Hashimoto 1978). Male *P. natans* in our collections might have been blown into the intertidal zone, which accounts for the low numbers caught at Wanliton where tidal pools are more isolated from the sublittoral waters at low tide than they are at Howan. The fertilized eggs must have been laid on the water surface and later sank to deeper levels. The survival of developing *natans* eggs might not be related to tidal levels, and the emergence time of adults showed a lower correlation with tidal rhythm (Fig. 1).

The 2 pontomyids may have used different cues for the time of their diurnal emergence. Potential environmental factors, such as times of sunrise, sunset, tidal rise and fall, as well as temperature are all related, and it is difficult to isolate the exact mechanism from field data. It is noted, however, that in *P. oceana* the mean time of emergence was highly correlated with, and usually prior to, the time of the following low tide (Table 1). It is thus postulated that whatever cues *P. oceana* may actually use, the adaptation is to emerge before low tide. This immediately raises the question of why *P. oceana* does not emerge before low tides during the day.

In the study area, spring low tide occurred during the day between April and September, whereas in other months of the year it occurred in the evenings (data from Anonymous 1992 1996). Thus, if spring tide is the proximate factor for the emergence of *P. oceana*, as in *C. tsushimensis* (Saigusa and Akiyama 1995), one would expect a massive emergence to occur during the day between April and September. However we have yet to collect *P. oceana* during daytime low tides. Moreover, in our laboratory cultures, which were not exposed to tidal fluctuations, all midges emerged at times after dark similar to those of natural colonies. Moreover, as tide is about 50 min later per day, the interval between the mean emergence time and the low tide also increases accordingly, within a semilunar cycle

<table>
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<tr>
<th>Species pairs</th>
<th>Site</th>
<th>Emergence time</th>
<th>Successful post-zygotic isolation</th>
<th>Ref.</th>
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<tbody>
<tr>
<td><em>Clunio aquilonius</em> and <em>C. tsushimensis</em></td>
<td>Japan</td>
<td>overlap</td>
<td>in lab, embryo inviable</td>
<td>Hashimoto 1969</td>
</tr>
<tr>
<td><em>Pontomyia natans</em> and <em>P. oceana</em></td>
<td>Taiwan</td>
<td>some overlap</td>
<td>not seen in the field</td>
<td>unclear</td>
</tr>
<tr>
<td><em>C. marinus</em> and <em>C. balticus</em></td>
<td>Europe</td>
<td>no overlap</td>
<td>in lab, fertile offspring</td>
<td>Heimbach 1978</td>
</tr>
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</table>

*Fig. 4.* Size frequency distribution of *Pontomyia oceana* larvae reared in the laboratory.
(Fig. 3). All the above suggest that tide is not a regulatory factor in synchronizing the diurnal emergence of _P. oceana_.

We consider light conditions to be an ultimate factor affecting the emergence of _P. oceana_ after sunset when the substrate might be cooler and the danger of predation lower. Actually, light conditions may well be the proximate factor cueing the diurnal emergence time, according to correlation analyses of environmental factors (Table 1). Additionally, photoperiod was the only controlled cycle in the laboratory; and midges emerged synchronously under the laboratory condition.

Difference in the timing of emergence has been found to be an effective mechanism for prezygotic isolation between the European marine midges _Clunio marinus_ and _C. balticus_ (Heimbach 1978). There is little morphological difference between the 2 species, which earlier was identified as a single species. Cross-breeding between the 2 species is possible under artificial conditions, and hybrids produce fertile offspring (Heimbach 1978). On the other hand, the 2 Japanese _Clunio_ spp. which emerge at similar time where they are sympatric, have developed postgametic isolation. Zygotes do not develop in crosses between _Clunio aquilonius_ and _C. tsushimensis_ (Hashimoto 1969). In this study, although an overlap in the emergence time between _P. natans_ and _P. oceana_ was observed (Fig. 2), no cross-breeding has been found in the field. Males of the 2 pontomyids are very different and easily distinguished from one another (Cheng and Hashimoto 1978). The differences among these 3 species pairs are summarized in table 2. It seems that secondary contact between species pairs has reinforced the evolution of reproductive isolation in these midges.

The biased sex-ratio toward females of _P. oceana_ observed in the laboratory (like _Clunio_ [Neumann 1966], but unlike _P. cottoni_ [Cheng and Collins 1980]) suggests that high frequencies of inbreeding may occur in nature due to low number of founders in a local population. Applying the formula of Karlin and Lessard (1986) to the sex ratio found in the laboratory (m: f = 191: 297), the expected number of founders in local populations of _P. oceana_ is estimated at about 4.3. If the approximation above is correct, most colonies of _P. oceana_ may have been started from a few midges which somehow drifted away from their original habitats.

Larval size measurements taken from the field, as well as from laboratory cultures (Fig. 4), indicate that the generation time of _P. oceana_ is about 30 d. A few larvae, however, may have an extended larval stage, about 45 d, under laboratory conditions (unpubl. data). It is unclear what causes this variation in larval development time, but there is little doubt that midges emerging 15 d apart in nature belong to the same population.

Males helping females to strip off their pupal skin was observed in some _Clunio_ spp. (Hashimoto 1957, Olander and Palmen 1968), but not in _P. oceana_. The presence of males, however, appears to be necessary for female eclosion. Due to the highly female-biased sex-ratio, most males might be engaged with a female. Thus, it would be advantageous for female pupae to wait for available males before eclosion.

If it can be assumed that the average life span of a male adult in nature is 2 h (2.4 h in the laboratory), the peak of accumulated male densities should occur 1 h later than the peak of emergence (Fig. 5). Thus a male is expected to emerge 1 h earlier than females in order to have the highest probability of finding mates. This is corroborated in laboratory cultures where males emerged, on average, 61 min earlier than females. Earlier male emergence has also been reported in _Clunio_ spp. (Hashimoto 1962, Neumann 1966) and _Pontomyia cottoni_ (Cheng and Collins 1980) and in some butterflies (Wiklund and Fagerstrom 1977).

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REFERENCES


海生搖蚊 (Pontomyia 屬) 之生活史研究

宋克義1 陳國芳1 曹俊仁1

本研究於民國81年及85年調查了臺灣南部兩種海生搖蚊Pontomyia oceana(大洋搖蚊)及P. natans(海洋搖蚊)的羽化週期。P. oceana僅在新月及滿月前後幾天出現，P. natans每天都採集得到，後者在傍晚出現，前者總是在日落後才羽化，P. oceana在夏天羽化時間較早，與冬天可相差達3小時以上，兩種搖蚊出現時間的重疊也由此因季節而異。P. oceana一天中羽化的時間不受潮水的誘發，而日出、日落可能是控制的關鍵，藉由羽化日期和時間的控制可使P. oceana都在早晚低潮前羽化。本調查期間未曾採集到P. natans的雌性成蟲，而P. oceana則可由採回成蚊所產的受精卵在實驗室中完成生活史。P. oceana的受精卵約4天孵化，經4期幼蟲、結蛹而後羽化，平均每時三十天。雌蟲比雄蟲早一小時孵化，羽化後可活動約兩小時，因此雌蟲開始羽化時正是雄蚊密度最高的時間。雄蚊的存在可促進浮上水面雌蛹的羽化。

關鍵詞：海生搖蚊，羽化，搖蚊科。

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