

## **Sympatric Species, *Clunio marinus* Hal. and *Cl. balticus* n. sp. (Dipt., Chironomidae), Isolated by Differences in Diel Emergence Time\***

Fred Heimbach

Physiological Ecology Section, Department of Zoology, University of Cologne,  
Weyertal 119, D-5000 Köln, Federal Republic of Germany

**Summary.** Two populations of *Clunio*, an intertidal one and a sublittoral one, were found sympatric in the Kviturdvikkollen near Bergen, western Norway. The times of eclosion and the immediately following reproduction were strictly separated in each species by only a few hours' difference. The emergence time of the intertidal population was strictly correlated with the afternoon low water of spring tides; the population resembles the Helgoland (North Sea) population in nearly all its morphological and physiological characteristics. The sublittoral population emerged nearly every day (during the summer) just after sunset, independent of the tides, with only a slight increase in number during the time of the first and last quarters of the moon. The sublittoral population is similar to the populations from the Baltic Sea, especially in its characteristics of emergence control and sinking of the suffused egg masses. Cross-breeding experiments with stocks from both populations resulted in an intermediary emergence time of the first filial generation which was never observed in the field. Therefore, one can conclude that the sympatric populations are perfectly isolated in nature and that they have the well-defined rank of two species. The intertidal population belongs to the well-known *Clunio marinus*; the sublittoral population together with well-known open-sea populations of the Baltic Sea are classified as a new species, *Clunio balticus* n. sp.

### **Introduction**

*Clunio* is one of the rare kinds of marine chironomids which settles in the lower midlittoral range of the intertidal zone, and in some locations even further down to a depth of 20 m in the sublittoral (Caspers, 1951; Neumann,

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1966). There are several populations on the coast of the European Atlantic and the North Sea with a larval habitat in the lower midlittoral (Neumann, 1976). The time of emergence and the reproduction which immediately follows is strictly correlated with the time of local low waters when egg-laying can take place on the exposed habitat. The larvae pupate only within a period of a few days every fortnight (semilunar rhythm of pupation) so that the imagos are ready to emerge 3–5 days later, in most locations on days with spring tides. The time of day for eclosion of the adults is correlated with the local time of low water, which recurs every 15 days at about the same time of day; it is based on gene-controlled properties of a diurnal rhythm of eclosion (Neumann, 1966). The adults are short-lived, most dying within about 2 h. After a mating flight on the water surface, the females can fasten their egg masses to the exposed substratum.

Contrary to this, *Clunio* populations in the Baltic Sea inhabit the sublittoral down to 20 m and are not exposed to tides. A semilunar rhythm was never observed. The daily time of emergence occurs immediately after sunset (Remmert, 1955; Neumann, 1966; Endrass, 1976a). After the mating flight on the surface of the open sea, the females lay their eggs on the water surface, from which the eggs sink down to the sublittoral region. This results from specific properties of the gelatinous matrix of the egg mass, which suffused immediately after it was laid (Endrass, 1976b).

In 1968, a new *Clunio* location was observed in the sheltered and shallow waters along the coast of central Norway, which showed a weak correlation of emergence times with the low waters of neap tides at about the first and last quarters of the moon (Koskinen, 1968). The adaptive significance of this smooth semilunar rhythm was not clear. Neumann and Honegger (1969) considered this *Clunio* location as a transitional type between populations of the lower midlittoral with strictly semilunar reproduction and the Arctic population of the upper midlittoral with a tidal rhythm of emergence.

Because striking morphological differences were not found between Atlantic and Baltic Sea populations (Strenzke, 1960; Palmén and Lindeberg, 1959; Koskinen, 1968; Olander and Palmén, 1968) and additionally, because stocks of all these populations could interbreed in the laboratory in spite of different physiological properties (Neumann, 1966, 1971), all the populations had been classified up to now as *Cl. marinus*. In spite of the fact that the Baltic midges tended to be bigger than those from the North Sea, Olander and Palmén (1968) also hesitated to give a subspecific rank to the Baltic Sea population because there had also been remarkable physiological differences between the different Atlantic populations. However, the special status of the Baltic Sea *Clunio* was again stressed by Endrass (1976b) according to unique ecophysiological adaptations.

As will be shown in this paper, two sympatric *Clunio* populations with different physiological and ecological properties were now found to exist at the Bergen location. One of them belonged to the Atlantic type and the other to the Baltic Sea type. On the basis of a complete reproductive isolation in the field, the taxonomic rank of the populations of the Baltic Sea could be clearly seen to represent a separate species, *Clunio balticus* n. sp.

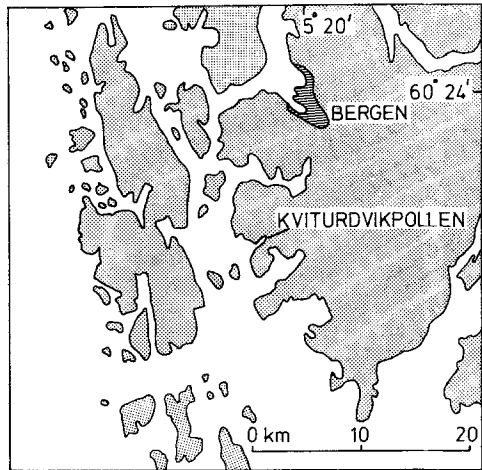


Fig. 1. Location of the *Clunio* populations near Bergen, western Norway

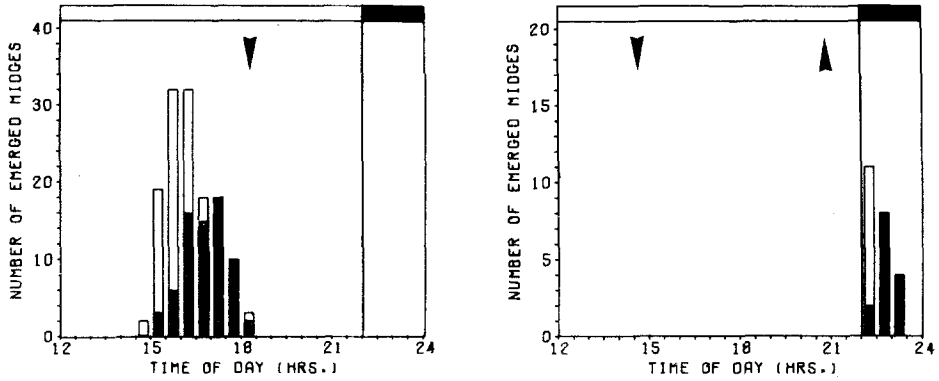
### The Biotope

At about 20 km south of Bergen in western Norway, *Clunio* was found in the Kviturdvickpollen (Fig. 1;  $60^{\circ}16'N$ ,  $5^{\circ}15'E$ ; Koskinen, 1968). This is a small, well-sheltered cove with the depth being roughly up to 6 m. The bottom is muddy for the most part (Jorde, 1966). The larvae of *Clunio* (up to several thousand per square meter) live among filiform algae. The two species, *Cl. marinus* and *Cl. balticus*, can be clearly distinguished only by the different times of their daily emergence (see below). In the upper sublittoral the areas of both species overlap. While *Cl. marinus* settles in the lower part of the narrow midlittoral and somewhat deeper down to the upper sublittoral, *Cl. balticus* lives in a range from the sublittoral fringe of the midlittoral down to the sublittoral parts of the cove.

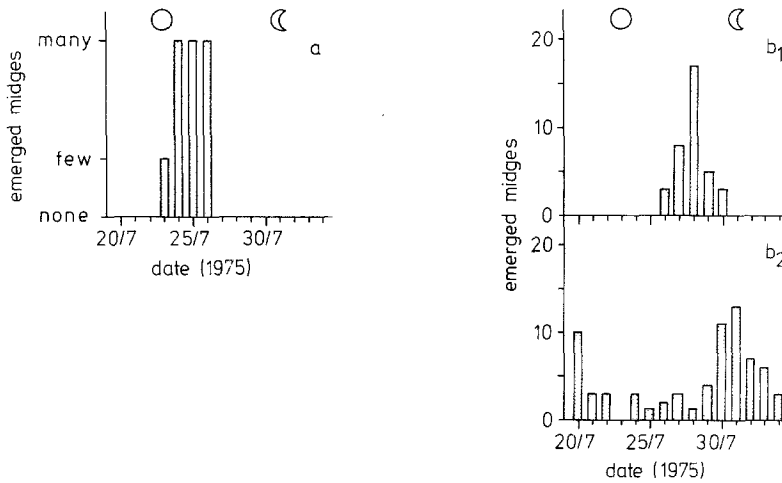
### Times of Emergence

The Bergen population of *Cl. marinus* emerged within the lower midlittoral only on days near spring tides (Fig. 3a), and then only 1–2 h before the afternoon low tide (Fig. 2a), so that the females were able to lay their egg masses on the exposed substratum. This emergence time corresponds well with the emergence times of other populations in central and southern Europe.

On the other hand, *Cl. balticus* emerged just after sunset (Fig. 2b); the females laid their egg masses on the water surface, not on any solid objects, and the eggs sank to the bottom. There was a slight semilunar-periodic synchronization on days near the neap tides. This semilunar synchronization was more distinctly expressed in shallower habitats than in deeper ones (Fig. 3b; compare with Koskinen, 1968). On days of increased emergence, the daily time of emergence coincided with the time of the incoming tide so that the sinking egg masses would drift into the cove. The sinking would normally take 7–8 min for about 5 m, but where there were currents it could take considerably longer.



**Fig. 2.** Daily emergence time of the intertidal population (*Clunio marinus*, left) and the sublittoral population (*Cl. balticus*, right) according to half-hourly registration on  $\frac{1}{4}$  m<sup>2</sup> in Kviturdvickpollen. White part of columns: male; black: female. The downward arrows mark the time of low water, the upward ones the time of high water. The darkness from sunset onwards is pointed out in black above the plots. Left hand plot 27/7/1972, right hand 4/8/1975



**Fig. 3a and b.** Numbers of daily emerged midges of *Chumio marinus* (a) and *Cl. balticus* (b) during a semilunar cycle of spring and neap tides in Kviturdvickpollen. Daily registration above an area of  $\frac{1}{16}$  m<sup>2</sup> each. *b*<sub>1</sub> about 25 m from the shore at about 1.0 m water depth (mean level); *b*<sub>2</sub> about 80 m from the shore at about 3.7 m depth. Fullmoon on 23/7/1975

This *Chumio* population is related to that from the western Baltic Sea, which was extensively described by Endrass (1976a, b).

In experiments with stocks of both Bergen species, the environmental factors controlling the times of emergence were analyzed (Heimbach, 1976). The timing mechanism of the *marinus* population corresponded with earlier results from the Helgoland population (Neumann, 1966, 1976). The semilunar-periodic synchronization is based on an internal semilunar clock mechanism controlled by a distinct phase relationship of tidal turbulence and the 24 h light-dark cycle; the diurnal time of emergence is based on circadian clock mechanisms with the 24 h light-dark cycle as zeitgeber. The control of the slight semilunar synchro-

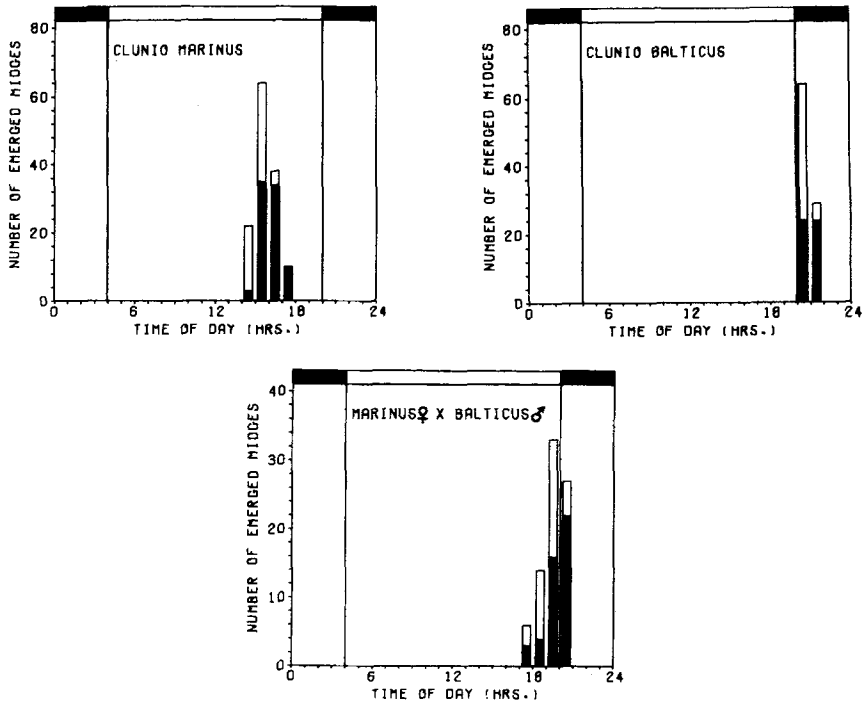


Fig. 4. Daily time of emergence in stocks of both Bergen populations (*Cl. marinus* and *Cl. balticus*) and in the first filial generation. Sum of 3 cultures in 8–9 days. Conditions: LD 16:8, 20°C. For further illustrations see Figure 2

nization of the *balticus* population is still an unsolved problem, as will be discussed elsewhere (Heimbach, in preparation), but according to the diel emergence time, an internal circadian timer is effective together with the light-dark cycle as zeitgeber too. Thus, the species-specific diel emergence time is based on different phase relationships between the circadian clock and the day-night cycle.

Cross-breeding between stocks of both species was possible when the cultures were bred under different zeitgeber regimes (phase-shifted 24 h light-dark cycles) in such a way that the imagos emerged at the same time of day in the laboratory (for further details of the method, see Neumann, 1966). The offspring of the two stocks emerged daily at a time which was intermediary between the times of emergence of the two parents (Fig. 4), and this result was identical in reciprocal crossbreedings. In nature, however, at the corresponding times (namely, the last 2 h before sunset) I could not find any imagos of *Chunio* during the several weeks of my field work. From this, one may conclude that hybridization between the two species normally does not occur in nature.

### Body Size

Various measurements of the size of *Chunio* were made in several populations (Strenzke, 1960; Neumann, 1966; Koskinen, 1968; Olander and Palmén, 1968).

**Table 1.** Measurements of *Clunio marinus* imagos (collected on 24/7/1975) and those of *Cl. balticus* (collected on 4/8/1975), both from Kviturdvikpollen near Bergen

Length (mm) of		Female body	Male body	Male wing	Hypopyg
<i>Clunio marinus</i>	m	1.90	1.83	1.39	0.51
	SD	0.23	0.18	0.10	0.04
	n	10	20	40	20
<i>Clunio balticus</i>	m	1.92	1.91	1.56	0.52
	SD	0.15	0.10	0.09	0.07
	n	14	21	39	12

There are differences, mainly in the body length of the midges of different populations, which are sometimes striking, especially between the imagos in the Baltic area and those in the Atlantic. However, as the measurements of the two species found at the same location near Bergen during the same season show (Table 1), no differences were observed between the species. This demonstrates the importance of abiotic factors for the increase in size of the insects, and above all, the importance of temperature, as was observed with several chironomids (Krüger, 1933) and even with *Cl. tsushimensis* (Hashimoto, 1968), which tended to be larger when reared at low temperatures. The slight differences between *Cl. marinus* and *Cl. balticus* from Bergen (Table 1) may depend on the fact that *Cl. balticus* larvae live in the sublittoral where the water temperatures may be somewhat lower than in the higher midlittoral, which is exposed to the air when the tides are low. The measurements from Koskinen (1968) also show this tendency. The midges of the spring generation tended to be larger than those of the summer generation, which had developed during the summer months with their higher temperatures. (These measurements are based only on midges of *Cl. balticus* and agree with my measurements.)

## Discussion

The taxonomic classification of the genus *Clunio* is not very easy because of the lack of precise morphological differences between the different geographical strains. Some earlier attempts at subdivision cannot be maintained (Strenzke, 1960; Palmén and Lindeberg, 1959; Neumann, 1966, 1971; Olander and Palmén, 1968). According to this, even the Baltic kinds were not ranked as a separate species, even though these insects were bigger than those of the Atlantic populations. However, as the present work shows, this could be due to lower temperatures in the locations further north. On the other hand, both Bergen populations illustrate the reproductive isolation of *Cl. balticus* from *Cl. marinus*. This isolation is not caused by sterility as, for instance, with *Cl. mediterraneus* (Neumann, 1966), but only by differences in the daily emergence time, which is sufficient for the sympatric life of the two populations. There is seldom a life cycle

as short as that of these insects, so that within the system of isolation mechanisms leading to a separate species Mayr (1963) did not classify the diurnal rhythms separately. However, in this case it is of as great importance as the other mechanisms of isolation.

The evolution of *Clunio* within the relatively young Baltic Sea is imaginable in that the midges, having drifted into the Baltic Sea from neighboring populations of the North Sea, adapted to the submersed way of life, changing the properties of their egg masses and their rhythm of eclosion from those dependent on the tides to those dependent only on the day-night cycle.

An evolution of the *balticus* type in Bergen itself seems to be improbable because the habitat overlaps that of *marinus* and thus an adequate spatial isolation for selection of both emergence types within the same location is not to be expected. It is more probable that *Cl. balticus* originated in the Baltic Sea and afterward settled in sheltered locations on the edge of the North Sea such as the coves near Bergen. In this part of the North Sea the diel emergence times of *Cl. marinus* at the time of the local spring low water and those of *Cl. balticus* just after sunset differ from each other to such an extent that the emergence times, and hence the times of reproduction, are completely separated from each other.

In summary, the morphology of *Cl. balticus* has been indistinguishable from that of *Cl. marinus* (described by Strenzke, 1960). The differences between them are limited to the physiological properties of the egg masses and the times of emergence. Whereas all *Cl. marinus* so far found were located along the coasts of the central European Atlantic and the North Sea (Caspers, 1951; Neumann, 1966, 1976), the populations of *Cl. balticus* were found in the Baltic Sea (Remmert, 1955; Palmén and Lindeberg, 1959; Neumann, 1966; Olander and Palmén, 1968) and at Bergen on the outer edge of the North Sea (Koskinen, 1968).

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