On the Salinity Tolerance of Eggs and Young Larvae of Phryganea grandis Linné (Trichoptera)

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INTRODUCTION

Most caddis larvae live in freshwater but several species also occur in brackish-water. At Tvärminne in the Gulf of Finland SILFVENIUS (1905) has found 24 species in the sea in a salinity range from 4.94 ‰ to 5.91 ‰. Some of the species were only found in the sea and not in the freshwater pools of the region. *Phryganea grandis* was one of these species. The same species was found by STAMMER (1928) in salinities from 2.56 ‰ to 7.15 ‰.

In a later paper SILTALA (SILFVENIUS) (1906 b) investigated the distribution of the caddis larvae in the Gulf of Finland and showed that the number of species increases considerably with a decreasing salinity. LINDBERG (1948) also found a greater number of species in the northern part of the Baltic where the salinity is lower than in the more salty southern part.

From water with a higher salinity there are a few records of caddis larvae. The larvae of *Philanisus plebejus* WALKER are fully marine, living in rock pools in the tidal zones of New Zealand and Australia (MCLACHLAN, 1883, 1887, 1891, HUDSON, 1904). In addition to this species there is at least one other species able to tolerate high salinities, viz. *Limnephilus affinis* CURTIS (SUTCLIFFE, 1960, 1961).

The present investigations have been carried out at Askö, an island in the archipelago of Trosa, in the northern part of the Baltic.

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Material and methods

In connection with quantitative and experimental investigations of the fauna of *Fucus vesiculosus* Linné in the Baltic, the author has studied caddis larvae of which a few species occur in comparatively great numbers in the rockweed-biotope in sheltered or slightly exposed localities.

In addition to *Phryganea grandis* and other caddis larvae also some other insects occur in the habitat. Larvae of *Chironomus spp.* are most common. In very sheltered locals *Deronectes depressus* Fbr., *Corixa* sp., larvae of *Sialis lutaria* Linné and larvae of different dragonflies are found comparatively infrequently.

In the shallow regions the animals may be exposed to salinity fluctuations in periods of extreme dilution and evaporation but these fluctuations are usually not of great importance. The part of the Baltic surrounding Askö has a low and stable salinity, varying between about 6 ‰ and 7 ‰.

The oxygen fluctuations in the habitat were not recorded. An oxygen deficiency lethal to the larvae would probably not occur.

Eggs

The egg-masses of the Trichoptera are of two different kinds, putty-like and jelly-like (Silfvenius, 1906 a). In the putty-like egg-masses the surrounding secretion swells slightly and forms a thin hide, which glues the eggs to the substratum. In the jelly-like ones it swells to several times its original size (Nielsen, 1942). The egg-masses of the Phryganeidae belong to the latter type. They are usually of a characteristic ring-form, but the egg-masses of most species within the family look very much alike (Silfvenius, 1906 a). An absolutely safe method of obtaining egg-rings from a particular species would be to isolate flies of this species in great aquaterraria. However, this has not been practicable. In order to make a safe classification of the species, some of the larvae which were hatched from eggs in the different test concentrations where larvae survived, were transferred to aquaria and reared to a size where a safe identification could be made. Moreover a great number of larvae bred from egg-masses collected at the same time as the egg-masses used in the experiments all proved to belong to *Phryganea grandis*. It is therefore presumed that all the egg-masses have been deposited by this species. The larvae have been identified after Silfvenius (1901, 1902, 1904), Ulmer (1903, 1909), Lestage (1912) and Hickin (1942, 1955).

The eggs of *Phryganea grandis* are always deposited submerged on floating leaves or on vertical plant-stems and leaf-stalks (Wesenberg-Lund, 1911). The caddis fly creeps down in the water on these to de-

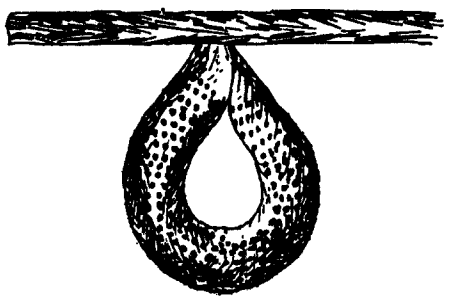

Fig. 1. Egg-wreath of *Phryganea grandis*. Full size. After WESENBERG-LUND, 1911.

posit its egg-string. The depth of deposition can be down to 1.2 metres, but this is rare.

The time of development of the eggs fluctuates (SILFVENIUS, 1906 a, WESENBERG-LUND, 1911, LESTAGE, 1921). The variation depends to a great extent on the temperature.

A large number of egg-rings were collected on the 25th and 26th of July 1966. The water temperature was 19—20°C at the moment of collection.

The stage of development of the eggs was roughly determined, and on the basis of this they were divided into two groups: eggs which exhibited a few cell-divisions, and far differentiated eggs where eyes and jaws could be seen. The development of the eggs was delayed by storing them in water from the biotope at a temperature of 5°C. At this temperature the egg-masses were kept until the start of the experiment.

Ten different test salinities were prepared by diluting 34 $^{0}/_{00}$ seawater with distilled water. The salinity was measured with a salinity temperature bridge. Fifteen egg-rings with early-stage eggs were divided into shares with 30 eggs in each. These eggs were placed directly in 2 series of 10 sterile petri dishes containing water with the following salinities: distilled water, 2, 5, 7, 10, 12, 15, 17 and 20 $^{0}/_{00}$ S. and a control dish with water from the habitat (6.4 $^{0}/_{00}$). The dishes were kept in complete darkness at a constant temperature of 15°C. The water in the dishes was changed once (9 August). In the beginning of the experiment observations on the numbers of hatched larvae were made every 12 hours, but after four days readings were taken once a day (except for the 21st and 22nd day after the start of the experiment). The experiment was started on 31 July and was interrupted after 52 days (21 September) or when all the eggs were hatched.

The late-stage eggs were tested in a similar manner: 20 egg-rings

were divided into shares of 50 eggs in each, and the shares were placed in petri dishes with 10 different salinities as described above. The control dish with water from the biotope was doubled. The water was changed once (5 August). The experiment was started on 31 July and was interrupted after 16 days (15 August), (Figs. 2, 3 and 4), and was read once a day. The mortality of the recently hatched larvae was also observed.

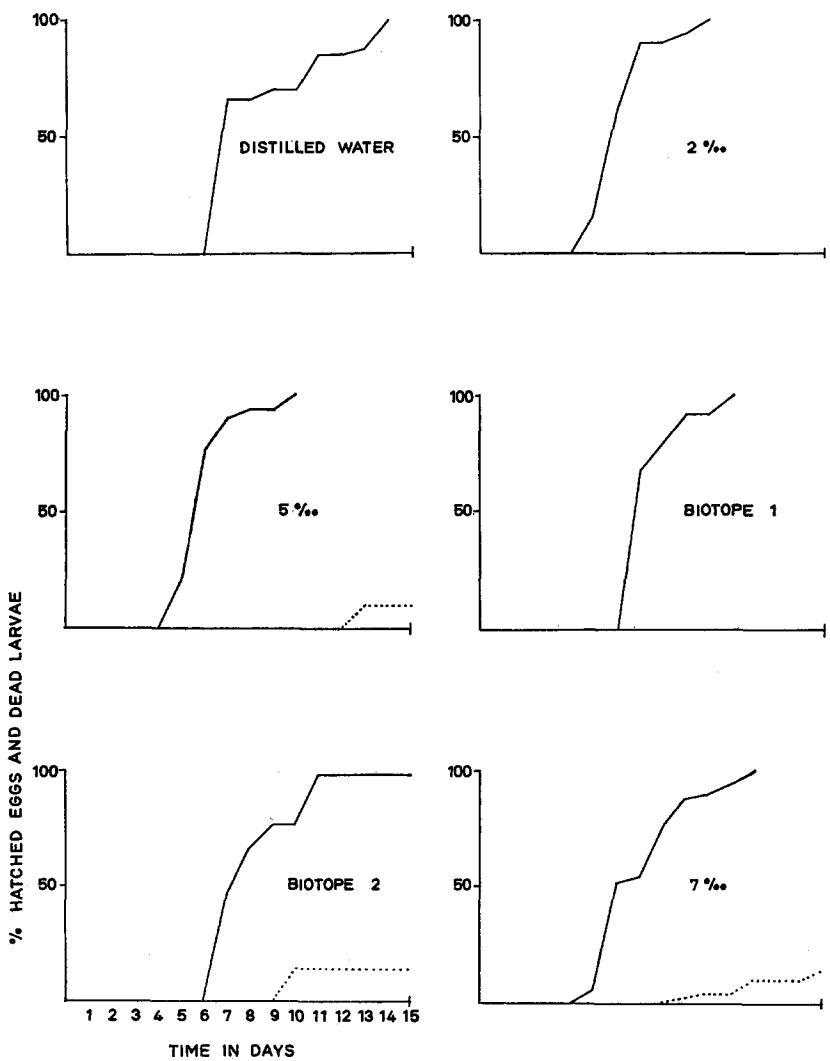

Fig. 2. The hatching of eggs taken in very late stages of development and the numbers of dead larvae in different salinities. ——% hatches eggs, - - - % dead larvae. n = 50. July 31st—Aug. 15th 1966.

In order to examine if the oxygen content varied in the dishes and if this fluctuation was so great that it might influence the result of the experiment, the oxygen content was tested 9 days (18 August) after the water change in the dishes containing the undeveloped eggs. In the same way the oxygen content of the water in the dishes containing

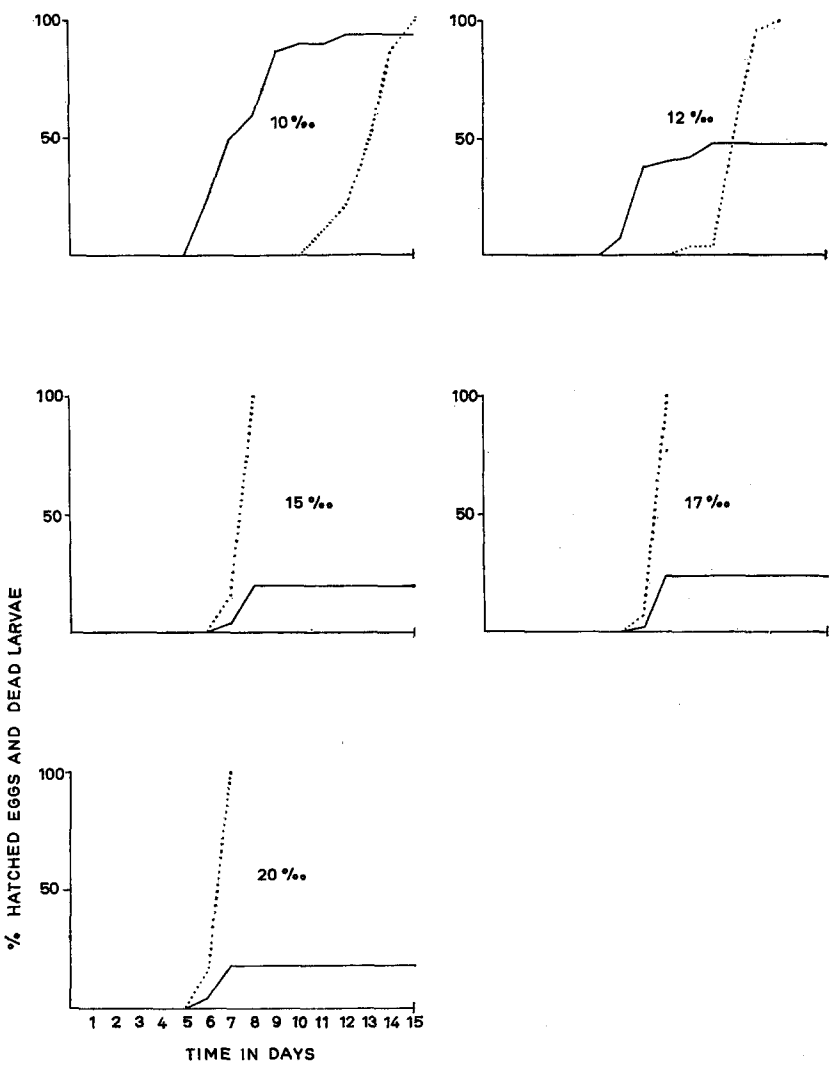

Fig. 3. The hatching of eggs taken in very late stages of development and the numbers of dead larvae in different salinities. —— % hatched eggs, - - - % dead larvae. n = 50.

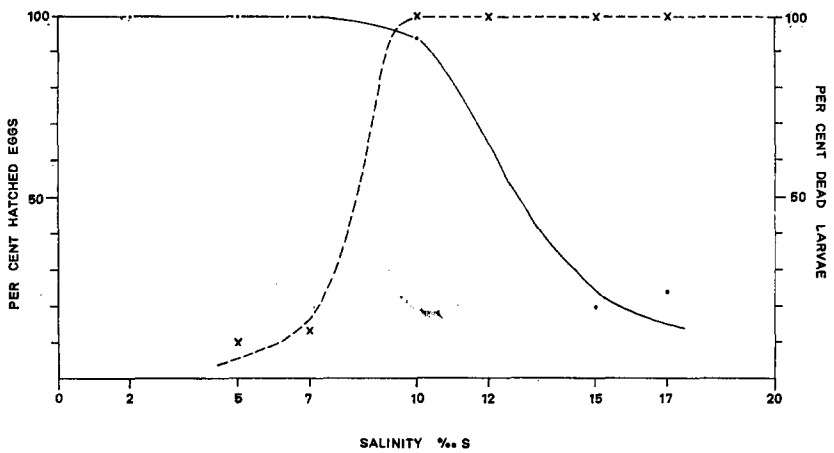

Fig. 4. The numbers of hatched eggs and dead larvae in different salinities. The eggs were taken from the biotope in a very late stage of development. The curves are fitted by eye.

the differentiated eggs was controlled. This check was made on the 5th of August or 6 days after the start of the experiment.

Larvae

The larvae used in the tolerance experiments were hatched from egg-masses collected on the 25th and 26th of July and kept in water from the biotope at a temperature of 15°C. They were taken a couple of days after hatching, when they had left the jelly-mass and were about 1.6—1.7 mm long and about 0.5 mm broad, and placed directly in 10 petri dishes containing water with the desired salinities. No larvae had built a case.

The experiments which were carried out at a temperature of 15°C, fall in two groups:

1. Larvae kept in constant darkness. The number of larvae was 30 in each dish.
2. Same condition but only 10 larvae in each dish.

In the petri dishes, case-building material consisting of fine algal filaments was placed. The animals were fed every day with meat of *Mytilus*. The water in the dishes was changed every third day with well-aerated water of the same salinity. The dishes were checked daily. The animals which did not move at a touch with a needle were regarded as dead and removed. The experiment started on 1 August, and was interrupted after 17 days (Fig. 5).

In the first experiment the oxygen content of the water in the petri dishes was controlled after three days.

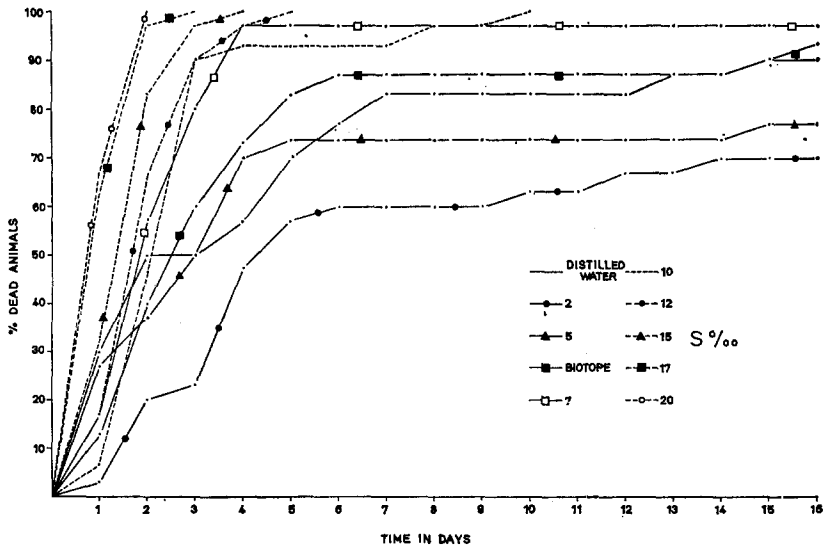

Fig. 5. Tolerance to salinity of young larvae of *Phryganea grandis*. The larvae were kept in darkness. n = 30. Aug. 1st—17th 1966.

Results and discussions

Salinity tolerance of the eggs

The table shows the percentage of hatching in experiments with early-stage eggs. In 5 ‰ S the experiment failed as the eggs were destroyed by fungi. Almost 75 % of the eggs hatched in distilled water. In 2 ‰ and in water from the biotope all the eggs hatched. A certain development of the eggs could be observed in 10 and 12 ‰ while the eggs which were kept in salinities of 15, 17 and 20 ‰ did not shown any development or a very weak one only. In concentrations above 10 ‰, however, no hatching occurred during the 52 days of observation.

In distilled water both the jelly-mass and the eggs absorb water and swell violently. The volume is on the whole unaltered in 2, 5, 7, 10 and 12 ‰. In higher salinities the jelly-mass shrinks considerably and the volume of the eggs diminishes and they get an indentation on each side.

The hatching of the late-stage eggs is shown in figs. 2, 3 and 4. In distilled water and in 2, 5 and 7 ‰ all the eggs hatched. 94 % of the eggs hatched in 10 ‰. Some of the eggs were infected with fungi in one of the two dishes with biotope-water. The percentage of

TABLE I

The hatching in per cent of newly laid eggs of *Phryganea grandis* in different salinities. July 31st—September 21nd, 1966.

S°/₀₀	Time in days										
	1	17	18	19	20	21	22	23	24	32	52
Distilled water	0	0	53	53	63			73	—	—	—
2,0	0	0	47	47	67			100			
Biotope	0	0	33	67	83			100			
7,0	0	0	50	67	67			97	—	—	—
10,0	0	0	0	0	0	0	0	0	0	0	0
12,0	0	0	0	0	0	0	0	0	0	0	0
15,0	0	0	0	0	0	0	0	0	0	0	0
17,0	0	0	0	0	0	0	0	0	0	0	0
20,0	0	0	0	0	0	0	0	0	0	0	0

hatched eggs was considerably reduced in higher salinities but even in the highest concentrations some hatching occurred.

The volume of the differentiated eggs is not influenced by the salinity to the same extent as the undeveloped ones.

It is possible that the time of development of the eggs is dependent on the salinity. That the eggs seem to hatch after different periods of time, however, primarily depends on the difficulty in determining the stage of development of the differentiated eggs.

KINNE (1964) reports that in many species, e.g. *Carcinus maenas* LINNÉ and *Gammarus duebeni* LILLJEBORG, the narrowest range of tolerance is during very early ontogeny. In *Phryganea grandis* the salinity tolerances of undeveloped eggs and young larvae correspond well, while larvae in the latest stage show a slightly higher tolerance (HAAGE unpubl.).

The reason why the differentiated eggs show a higher tolerance than newly deposited may be that the proporties of the egg-membranes or the jelly-mass change during development. The difference in volume change indicates this. The results show that the eggs close to hatching have no difficulties in tolerating an occasional increase of the salinity.

In figs. 2, 3 and 4 the mortality of the recently hatched larvae is also accounted for. In 15, 17 and 20 °/₀₀ S the larvae die almost immediately after hatching, and before they have left the jelly-mass. All the larvae survived in distilled water and in 2 and 5 °/₀₀. The mortality was moderate in one of the two dishes with biotope water and in 7 °/₀₀, while all the larvae in 10 and 12 °/₀₀ were dead after a couple of days.

The oxygen fluctuations in the dishes keeping the undeveloped eggs ranged from 8.1 to 8.7 mg O_2/l and in the dishes keeping the well-developed ones from 6.9 to 7.5 mg O_2/l. The variations are so small that they can be neglected.

Salinity tolerance of the young larvae

It is impossible to keep several larvae together in a limited space, as they attack and eat one another. SILTALA (1907) thinks that most species of *Phryganea* are carnivorous. WESENBERG-LUND (1943) notes that *Phryganea grandis* predominantly is a predaceous animal which eats both caddis and other insect larvae. SMIRNOV (1962) states that the larvae for the main part feed on higher plants but that they readily eat animal food, and SPANDL (1923) is of the same opinion.

To avoid cannibalism the larvae were fed. They willingly ate meat of *Mytilus* while algal-filaments were rejected. In spite of this the larvae attacked and ate each other, particularly during the first days of experiments with the lower salinities. This is no doubt due to the large number of larvae which makes contact between each other more frequent. When the number of animals diminished, the caddis had greater possibilities to withdraw on an attack. As it is impossible to know if a certain larva was dead or dying before it was swallowed, these larvae are included in the n-value and thus constitute a source of error. In the higher salinities, 12 to 20 $^0/_{00}$ this is, however, eliminated as the larvae in these concentrations do not eat at all. In 10 $^0/_{00}$ the number of animals eaten was very small.

Fig. 5 shows the results of the first tolerance experiment. At 20 $^0/_{00}$ all larvae died within two days. At 17, 15, and 12 $^0/_{00}$ all animals died within three, four and five days respectively. The tolerance in 10 and 7 $^0/_{00}$ was very much alike. However, some of the larvae were able to tolerate 7 $^0/_{00}$ during the whole experimental period, while all the larvae in 10 $^0/_{00}$ were dead within 11 days. The animals would stand distilled water very well and the mortality coincided on the whole with that of the biotope series. The mean value of the animals lost due to cannibalism in salinities less than 10 $^0/_{00}$ was about 25%.

The second tolerance experiment, with 10 larvae in each salinity gave results corresponding with the above mentioned experiment.

The control of the oxygen content in the water of the petri dishes after 3 days in the first tolerance experiment, indicated a variation from 8.2 to 9.6 mg O_2/l. The control was only made in the dishes where the animals were still alive. The lowest oxygen content occurred in the biotope water. This probably is due to a larger amount of microorganisms. The fluctuations are so small that they are of no importance for the result of the tolerance experiment.

Larvae of *Phryganea grandis* and other case-building caddis pro-

duce with irregular intervals a flow of water through the case by undulatory movements of the abdomen. Both larvae with and without cases make this movements. High water temperatures and poorly aerated water raise the frequency of ventilation movements (VAN DAM, 1937, FOX & SIDNEY, 1953 and PHILIPSON, 1955). The respiratory movements of the caddis in the dishes, with short periods of undulatory movements and long pauses speaks in favour of the sufficiency of the oxygen contents.

In the higher salinities the tolerance of the larvae coincide well with that of recently hatched larvae (Figs. 2 and 3). In the lower salinities the series differ. This is partly due to the fact that the animals were feeding on each other. After a few days, however, cannibalism became rare or did not occur at all. Survival is highest at concentrations lower than 5 $^{0}/_{00}$ and in water from the biotope.

The experiments indicate that the limit of the salinity tolerance of larvae of *Phryganea grandis* in the first stages of their development is about 7 $^{0}/_{00}$ but that the animals are able to tolerate a slightly higher salinity during a short period. The larvae may consequently be considered as stenosaline (KINNE, 1964).

The distribution of the trichopterous larvae indicates that the caddis worms, with few exceptions, are not able to tolerate salinities over 7—10 $^{0}/_{00}$ at least during longer periods. BUTLER & POPHAM (1958) suppose that a salinity of about 8.5 $^{0}/_{00}$ is critical to many freshwater insects.

The temperature can modify the effects of the salinity and enlarge, reduce or shift the salinity range of an individual (KINNE, 1964) GRESENS (1928) has studied the salinity resistance of some freshwater organisms and found that a decrease of temperature increases the time of survival. According to unpublished experiments the same holds good for *Phryganea grandis* larvae in their final instar. A decrease in the temperature of about 10°C considerably reduces mortality in 15 and 20 $^{0}/_{00}$ S. The great importance of the temperature on the larval and pupal development in different salinities has been pointed out by SUTCLIFFE (1960).

Case-building capacity of the larvae in the different salinities

As soon as the hatched larvae have left the jelly-mass they start to build cases. At this work the larvae can use very different materials (WESENBERG-LUND, 1911). The larvae had no plant-material in the storing aquaria. They then built cases of mud-particles in the water or of parts of the muddy jelly-mass they had left. Very often the larvae built spruce cases from heads and legs of their eaten or dead equals.

In the tolerance experiments there were fine algal filaments in the

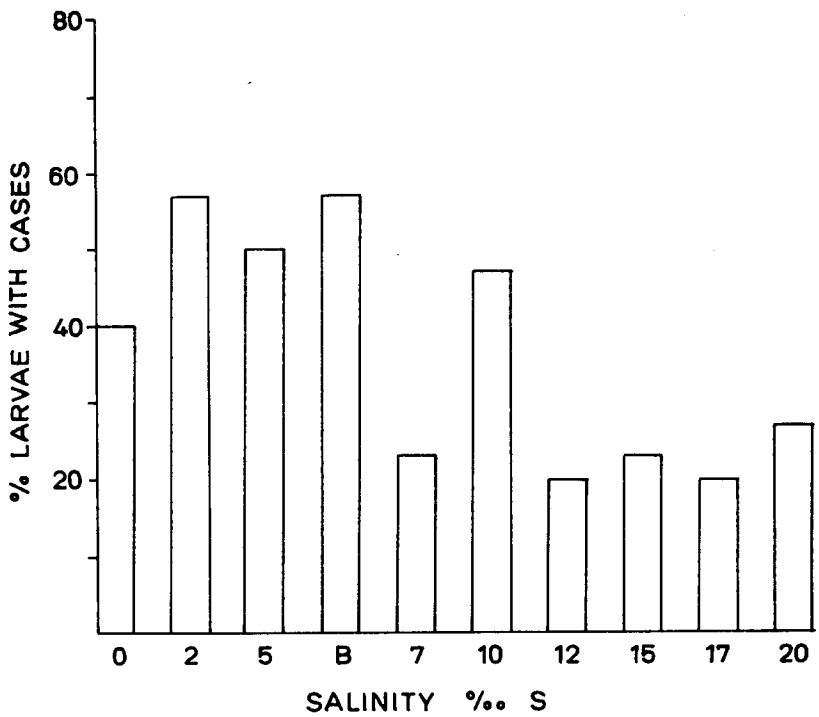

Fig. 6. Numbers of case-building larvae in different salinities one day after the start of the experiment. The larvae were kept in darkness. B = biotope. n = 30. Aug. 2nd 1966.

petri dishes. The larvae used these in case construction. The animals built cases in all the salinities, but the number of caddis able to carry out a case-building diminished in the higher concentrations. In fig. 6 the number of larvae with cases one day after the start of the experiment can be seen. The figure shows the result of the first tolerance experiment.

In the lower salinities the animals bit off fragments of the algal filaments of almost equal size, and joined these to a typical spiral band. In salinities of 12—20 $^{0}/_{00}$ the cases were very badly built, the algal fragments were not of the same size and they were loosely and unevenly joined so that the cases showed holes and they easily fell to pieces.

Summary

The salinity tolerance of eggs and recently hatched larvae of *Phryganea grandis* Linné (Trichoptera) from brackish-water in the

Baltic Sea was investigated. Freshly deposited eggs will not develop to hatching in salinities above 7 ‰. More mature eggs will hatch even in 20 ‰ but the larvae die soon after emerging. Larvae have the same tolerance range as freshly deposited eggs (distilled water to 7 ‰) in accordance with the actual distribution of the species. The high tolerance of mature eggs is probably due to changes in the egg-membranes or in the jelly of the egg-masses.

Case building becomes abnormal in salinities above 10 ‰.

Résumé

La tolérance de sel des oeufs et des larves récemment écloses de *Phryganea grandis* Linné (Trichoptera) de l'eau saumâtre de la Baltique a été examinée. Des oeufs nouvellement pondus ne se développeront pas jusqu'à éclosion dans une salinité de 7 ‰. Des oeufs plus mûrs sortiront de l'oeuf même dans une salinité de 20 ‰, mais les larves meurent bientôt. Les larves ont la même limite de tolérance que des oeufs nouvellement pondus (de l'eau distillée de 7 ‰), conformément à la distribution actuelle des espèces. La haute tolérance des oeufs mûrs est probablement conditionnée par des changements dans les membranes des oeufs ou dans la gelée des masses d'oeufs. La construction des fourreaux devient anormale dans des salinités au-dessus de 10 ‰.

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