Freshwater Biological Association

FBA Translation (New Series) No. 71

Title: Comparative investigations on the respiratory physiology of euryhaline gammarids with special reference to salinity adaptation.

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Reference: Helgoländer wiss. Meeresunters. 23, 485-534.

Original language: German

Date of publication of original: 1972

Translator: P.L.Nock

Date of publication of translation: 1974

Number of pages of translation: 62

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COMPARATIVE INVESTIGATIONS ON THE RESPIRATORY PHYSIOLOGY OF EURYHALINE GAMMARIDS WITH SPECIAL REFERENCE TO SALINITY ADAPTATION.

by H.-P. Bulnheim.

(Helgoländer wiss. Meeresunters. 23, 485-534, 1972).

Translated by P.L. Nock

In the region of the North Sea and the Baltic there occur several species of the genus Gammarus, very closely related to each other, which partly also colonise estuaries and other neighbouring brackish waters. To the species distributed along the German seacoast there belong Gammarus locusta (L.), G. oceanicus SEGERSTRÅLE, G. salinus SPOONER, G. zaddachi SEXTON and G. duebeni LILLJEBORG. Although morphologically separated only through very few characteristics, these shrimp species are revealed in a partly ecological respect to be significantly differentiated from one another. These differences are particularly evident in tolerance and preference compared with regard to the salt content of the external medium, but also in some other characteristics.

On account of small structural differences, a sure systematic demarcation for some of the species mentioned has been in question for a long time. Apart from G. duebeni, whose taxonomic individuality remained undisputed, G. zaddachi, G. salinus and G. oceanicus were next considered as a separate species under the title G. zaddachi (SEXTON 1912), later as three subspecies - G. zaddachi zaddachi, G. zaddachi salinus and G. zaddachi oceanicus (SPOONER 1947, 1951, SEGERSTRÅLE 1947). These subspecies were finally elevated to the rank of genuine species by KINNE (1954) after significant criteria for their genetic individuality had been discovered. The separation of these three as sibling species of similar form has not only been complicated by their morphological similarity, but also through the fact, that their environments partially overlap.

On the basis of a taxonomic revision of the G. locusta group, the existence meanwhile of further sibling species has been established by STOCK (1967). Thus next to G. locusta, which previously had been frequently confused with the above-mentioned forms, there could be defined four hitherto unrecognised species, which occur in different European sea areas, and amongst these

G. inaequicauda STOCK, which had recently been found in the Baltic (JAŻDŻEWSKI 1970).

In view of the fact that for these members of the family Gammaridae one is dealing with groups of species which are very closely interrelated, there arises the question as to how far they also differ amongst themselves through physiological characteristics. The discovery that the more or less clearly ecological differentiation which exists amongst the individual Gammarus species and is correlated with metabolic and physiological differences, has already been made by a series of works on the osmotic and ion-regulating capacity (B EADLE & CRAGG 1940, KINNE 1952, WERNTZ 1963, LOCKWOOD 1961, 1964, 1965, 1970, SUTCLIFFE 1967a, b, 1968, 1971 a, b, SUTCLIFFE & SHAW 1967, 1968 inter alia) as well as some data obtained through comparative experiments on the oxygen requirements (LÖWENSTEIN 1935, SUOMALAINEN 1958, ROUX & ROUX 1967, ROUX 1972) of different representatives of this genus in marine, brackish and freshwater environments.

The comparative respiratory and physiological experiments already made on the five euryhaline species Gammarus locusta, G. oceanicus, G. salinus, G. zaddachi and G. duebeni provide an experimental contribution to the further clarification of this problem. In view of the productive biological significance due to these amphipods within the marine littoral on the basis of their individual sovereignty, it would be interesting to obtain information about their respiratory intensity, in order to be able better to evaluate their role in the material and energy cycle.

The respiratory measurements carried out within the framework of this experiment were occupied with the relationships between oxygen consumption and body size depending on salinity. They also had the object of determining the variations in metabolic intensity after an abrupt change in the salt content of the external medium, and to establish the period of time for the process of adaptation. As the experiments were carried out polarographically in a testing plant with continuous flow-through, and the method which was applied permitted continuous recording over prolonged intervals, there could also be carried out~~x~~ comparisons between metabolism at rest and under activity, and the alterations of oxygen consumption during the process of moulting could be measured.

MATERIAL AND METHODS.

Experimental Objects.

The above-mentioned five species served as experimental objects, and it was possible to rear them all in the laboratory. The initial population of Gammarus duebeni originated from the Elbe estuary near Müggendorf, that of G. zaddachi from the river mouths of the Weser and the Elbe. G. salinus was taken on the rocky shore at Heligoland (north-east sandbank), G. oceanicus in the entrance to the Kiel(near the mouth of the Schwentine) and G. locusta in the sandbank region of List (Sylt), as well as in the outer harbour of Heligoland. Neither for G. locusta nor for G. zaddachi could population-based differences be established with regard to the oxygen requirement; the corresponding measured values are therefore not separately specified. The determination of the individual species followed the identifying keys of KINNE (1954) and STOCK (1967).

All the Gammarus species were placed in large tiled basins with a water volume of c. 100-115 l. or in smaller aquaria with bottom filter cultures, which were occupied by the freshly-caught specimens which had already been identified. G. duebeni and G. salinus proved relatively easy to rear through a low mortality rate, while the other three species showed a higher mortality and also could not be brought to multiply continuously. The method of culture largely corresponded to that which has been applied for the breeding of G. duebeni and has already been described in another connection (cf. BULNHEIM 1969, 1972). As food, the shrimps were given algae (Enteromorpha, Ulva), enchytra~~eus~~ids and roast mussel (mantle, glands of the small intestine and reproductive tissue). The water was constantly aerated and from time to time partially or completely renewed. The temperature of the culture stood at 15 ± 2°C. Greater discrepancies in the overall temperature (15°C) arose in general only for a short period with extreme outer temperatures in winter and high summer.

The individuals which were used for the respiratory experiments originated as a rule from the successive generations of the specimens taken in the wild (apart from some measurements on G. zaddachi and G. oceanicus). In order to have at our disposal over a long period of time experimental animals which had been identically handled, the individual Gammarus species were reared from the start in two salt content stages (10‰ and 30°/oo). In contrast to G. locusta, G. salinus, G. oceanicus and G. duebeni, the culture of G. zaddachi was least successful in media of 30°/oo, so that no continuous cultures could be bred in this salt content stage.

After completion of the respiratory measurements, the shrimps were drugged in a 0.1 percent solution of MS-222 (firm of Sandoz, Nuremberg), in order to determine their sex as well as to establish their fresh weight. Before being weighed, the gammarids were freed of adhering water by being dabbed with filter paper, especially of the liquid remaining between the legs and the gills. In all given cases the oxygen consumption values were obtained on the wet weight. As was established through the comparative weighing of living and dried animals, the water content amounts to 78% for G. duebeni and 80% for G. locusta. These data make possible an approximate subsequent conversion of the measurements into the dry weight. It should finally be noted, that of the females only those specimens, whose marsupium contained neither eggs nor embryoes were selected as experimental animals.

The production of the individual salt content concentrations took place through dilution of sea water with mains water (alkalinity: p-value 0.35-0.40, m-value 3.8-4.3 mval./l.) and through the addition of commercial sea salt. Salinity was determined by the aereometric and osmotic methods.

METHOD.

As has already been indicated in the introduction, the pO_2 was measured on the polarographic principle with the help of platinum-silver electrodes. The electrochemical method of measuring oxygen has in recent times achieved additional significance, since in contrast to other conventional methods (eg. manometry, WINKLER oxygen determination, SCHOLANDER'S method), since it is marked by a smaller expenditure of effort, good reproduction of the measured values and the advantage of a continuous measuring process in long-term experiments.

For the present investigations a stabilised membrane-covered all-glass platinum electrode (after GLEICHMANN & LÜBBERS 1960) was used, in the modification as a spherical surface electrode (LÜBBERS & WINDISCH 1963). This electrode, constructed on the CLARK principle, although substantially improved, is separated from the medium to be measured by a watertight, although oxygen-permeable, 25-μm. thick Teflon membrane. It consists of a platinum wire cathode (measuring electrode) and a silver anode (supply electrode), which was plated with silver chloride to raise the stability of measuring (cf. RING et al. 1969).

The cathode, of 200-μm. thick platinum wire welded into glass free of air bubbles, lies in the distal spherically-shaped part of the electrode, which fits into the electrode-covering receptacle - which is likewise of glass - and is secured there. The cathode is connected for conductivity with the anode lying beneath the head of the electrode by an electrolyte solution (0.5 m. KCl at the chloridised anode). A thin film of cellophane (~12 μm) lies between the cathode and the Teflon membrane as a stabilising partition, so that a constant, unaltered diffusion space is provided. At the base of the electrode lies the analysis chamber, and extension of the glass capillary system. It contains a small glass stirrer, in which an iron plate has been welded. A magnetic stirrer attached beneath the electrode sets the rod in rotation (370 U/min.). The turbulence arising from this ensures a uniform diffusion of the oxygen out of the measuring solution onto the platinum surface.

The physico-chemical processes, which take place at the cathode when voltage is turned on, have been described amongst others by GLEICHMANN & LÜBBERS (1960). The molecular oxygen diffusing at the platinum surface is charged with electrons; there the reduced oxygen reacts with the hydrogen ions of the water in several steps with the formation of hydroxyl ions.

The current flowing on the reduction of oxygen at the cathode is dependent on the number of diffused oxygen molecules. If the voltage applied at the electrode is large enough to reduce all oxygen molecules at once, the reduction current is proportional to the oxygen pressure in the solution. The voltage curve (polarogram) is decisive for the measurements in gas analysis. First of all the reduction current increases wich increasing voltage, and indeed right up to a region (about 600-900 MV) in which the amount of current remains constant with further increase of voltage. As the current is a function of the oxygen concentration in this so-called region of current diffusion limit, this has been selected as the area in which to work. With the application of electrodes whose anode shows a covering of silver chloride, it seemed best to conduct the measurements at a polarisation voltage of 800-900 mV.

The measuring arrangements for the present question consisted of an analysis section with one or two ~~cohering~~ sintered glass vessels respectively for equalising the medium with oxygen from the air, a respiration chamber and a capillary system, which made up the connection between ~~cohering~~ sintered glass bottles, respiration chamber and electrode ~~produced~~, and of one section for measurements. The analysis section was in a thermostatic water bath, which was regulated at the experimental temperature of 15 ± 0.05°C. With the help of a high accurate performance pump (Dauerinfusionsapparatur Unita IIb, Firma Braun, Melsungen) the medium to be measured was delivered out of the ~~cohering~~ sintered glass bottle at a constant spped to the electrode, either on a direct route on taking up the empty value, or via the respiration chamber if this were occupied by an experimental animal. As Fig. 1 shows, the direction of the water current within the flow apparatus could be altered by means of several three-way taps ~~which had been~~ mounted within the capillary system. Through the installation of a second pump, which had been connected to the three-way tap lying behind the respiration chamber, the empty value could also be taken intermittently in long-term measurements after switching the three-way tap, without the necessity of taking the experimental animal out of the breathing chamber. Respiration chambers of different volumes were used according to the size of the individual. A strip of synthetic gauze was inserted in the respiration chamber to damp down locomotive activity; as the experimental animals settled on this, they came largely to rest as a rule after some time, on the basis of their strongly thigmotactical behaviour.

The electrode was connected to the measuring apparatus after its preparation (covering, electrolyte filling, sealing, etc.) and functional testing (Combi-Analysator of the firm Eschweiler & Co., Kiel). This delivers the polarisation voltage, strengthens the current flowing through the circuit and allows the measured values to be read; these are continually recorded by means of a compensation curved recorder (model "Servogor", firm of Metrawatt, Nuremberg). The latter was connected via an appliance to zero pressure on the Combi-analyser.

With the calibration of the electrode, the entry of gas mixtures with a defined oxygen content from atmospheric air saturated with water vapour was dispensed with, since small gas bubbles often formed at the electrode membrane after liquid had been later channelled through; the removal was only occasionally successful after a good deal of trouble. The zero setting was made with the help of a freshly-applied solution of sodium dithionite. Oxygen dissolved in the water is chemically bound through the addition of sodium dithionite. The solution was poured into a filler set in front of the electrode and connected with the capillary system by a three-way tap. As the calibration curve shows a linear course under constant temperature, the determination of a second calibration point is sufficient. This was determined with temperate, air-saturated water, which was subsequently passed by the electrode in a constant flow.

The stabilised all-glass platinum electrode in use (producer: firm of Eschweiler & Co., Kiel) shows great sensitivity, good reproduction and is rapidly installed (c. 20-30 sec.). The average error of a unit of measurement amounted to ± 0.5% (GLEICHMANN & LÜBBERS 1960); the minimum reading-off accuracy on the recorder lies at about 0.3 Torr. A satisfactory measurement stability in the electrode could be shown by its uninterrupted presence in the flow-through apparatus in general over a period of 2-3 weeks, exceptionally also up to about 4 weeks, before a new covering with the previously mentioned films and a new electrolyte filling had to be made. Next to other factors, the quality of an electrode depends primarily on the care which is devoted to the manual installation. As an example of particularly good stability, a range of measurements ~~should be~~ can be taken, in which the discrepancy within 4 days amounts to only -0.2% from the nominal value and within 6 days to -0.5%, with special regard to alterations in air pressure. An electrode drift is at the most counteracted through a negligible alteration in the polarisation voltage.

The experimental medium was filtered before use, sterilised by means of an ultraviolet lamp and filled into a supply flask. This was connected with a bottle with a standard level, through which the water level in the sintered glass ~~cohering~~ bottle could be held at a constant level. In the experiments on the effect of an abrupt alteration of salt content, two ~~cohering~~ sintered glass bottles were installed with media of different salinity, out of which, according to the positioning of the stop-cocks, air-saturated water of the one or the other salt concentration was pumped through the capillary system (cf. Fig. 1).

The experimental animals were in general placed in the respiration chamber after a one-day period of starvation, where they remained, according to the problem in question, for varying lengths of time, in any case for at least three hours, until arriving at a constant value. Before and after a measurement, the empty value was taken as control, and also intermittently for long-term experiments.

The evaluation of the measurement data which were continuously registered on the compensation recorder, followed at intervals of about 20, 40, or 60 minutes. As far as the respiratory curves showed larger oscillations, the results were derived from graphically determined average values.

With the help of a barograph, the actual air pressure was shown continuously, which should be borne in mind for the calibration.

Calculations.

For the calibration and the conversion of the variations measured for the pO_2, in units of volume, the following relationships are valid, which are set out as follows. They are governed by the laws on the solubility of gases in liquids.

The solubility of a gas in a soluble medium is proportional to its pressure or its partial pressure respectively, and inversely proportional to the temperature as well as the content for the dissolved substanee. The oxygen pressure in water saturated with atmospheric air thus corresponds to the partial pressure of the oxygen in the open air. With special reference to the ~~steam~~ water vapour pressure of the water, through which the proportional parts of the atmospheric gases are altered depending on the temperature, there results for the calculation of the oxygen pressure (p02): pO_2

$$pO_2 = 0.2094\ (p_a - p_w) \tag{1}$$

(0.2094 = oxygen partial pressure of dry air related to 1 atm; p_a = actual air pressure; p_w = partial water vapour pressure at the temperature of the experiment).

The values for the ~~steam~~ water vapour pressure of water depending on temperature have been collected and tabulated by OPITZ & BARTELS (1955). The oxygen pressure in stabilised water is determined through the actual air pressure and ~~steam~~ water vapour pressure, but not through the concentration of dissolved substances. Hence water of varied salinity shows, under otherwise identical physical conditions, the same oxygen pressure; the volumes are however different on the basis of their dependence on the concentration of dissolved substances.

With the conversion of the measured pO_2 differences in units of volume, the Bunsen absoption coefficient (a) of oxygen must be taken into account. This shows the volume of a gas obtained at 0°C and 1 atm. pressure, which will be dissolved in 1 cc. of liquid at a stated temperature and a stated pressure. The following formula arises for the BUNSEN absoption coefficient (cf. EUCKEN & WICKE 1958):

$$a = \frac{L \cdot 273.15}{273.15 + t} \tag{2}$$

(L = OSWALD solution coefficient; t = temperature of the experiment in °C).

The OSWALD solubility coefficient is defined through the concentration content (c) of the gas contained in the liquid and in the gas mixture above it. In the case under consideration we have:

$$L = \frac{c_{O_2}\ (\text{water})}{c_{O_2}\ (\text{air})} \qquad (3)$$

The data of GREEN and CARRITT (1967) asre the basis of the solubility of oxygen in water of varying salt content. By means of the absopption coefficients, the oxygen concentration (VO_2) in dependence on the oxygen partial pressure (pO_2) and the total pressure of the gas mixture (normal conditions for air P = 760 Torr) is caluulated as follows:

$$VO_2 = \frac{a.pO_2}{p} \qquad (4)$$

For conversion of the measured alterations of the oxygen pressure in units of volume (cc. O_2) there results with reference to the water volume supplied (in cc. per unit of time) for the oxygen consumption:

$$VO_2 = \frac{a.\Delta\ \text{Torr}\ .v}{p} \qquad (5)$$

(v = volume of the liquid supplied; Δ Torr = measured alteration of the oxygen pressure).

The oxygen consumption can then be converted into units of weight (obtained for the fresh or dry weight of the experimental objects).

RESULTS

The respiratory metabolism comprises the oxidative processes taking place in the organism, which serve to create energy for the maintenance of the life processes and the synthesis of body materials. The oxygen consumption as an indirect mass for the interchange of materials and energy is dependent on numerous factors of an exogen~~ic~~ous and endogen~~ic~~ous nature, partly standing in interaction. The analysis of the effect of all unit factors, as well as the illumination of their complex interplay, presents an extraordinarily extensive task, which can only be solved in numerous single steps, a task to which the present investigations, primarily effective in an interspecific comparison, should also afford a contribution.

Dependence on size and salinity

The problem of the dependence on size of the respiratory intensity for the individual Gammarus species formed the first central point of the experiments. Numerous experiments on representatives of different poikilothermic animal groups, as well as on crustaceans (cf. WOLVEKAMP & WATERMAN 1960) have ledto the realisation that the oxygen consumption represents a ~~size~~ value which lies mostly in the region between surface area and body weight proportionality.

The application of the allometric function offers a significant mathematical description of the relationships between body weight and metabolic size:

$$y = a.x^{b} \tag{6}$$

In this formula, y = the metabolic size, x = the animal weight, while a and b represent coefficients in logarithmic form. The allometric function is the equation of a straight line:

$$\log y = \log a + b.\log x \tag{7}$$

With the graphical representation of the basic metabolic rate ~~amounts~~ as a function of the weight in a double-logarithmic coordinate system, one receives a straight line, on which or in the vicinity of which, respectively, there lie the measured values. The tangent of the angle of ascent of these ~~regression~~ straight line~~s~~ regressions is determined by the exponent b, the regression coefficient. Its value thus identifies the dependence of respiratory intensity on body size. It varies mostly between 0.66 (surface proportion) and 1.0 (weight proportion). The parameter a represents the metabolic size of the selected unit of weight (in this case 1 g.) and indicates the average of the regression line with the ordinate axis. Together with the regression coefficient, it forms an appropriate standard for specific comparison of species or groups. Through the determination of both these parameters there is given the possibility of calculating the respiratory volumes of any other class of weight.

It is imperative to have under consideration a wide range of weights for the most exact possible determination of both values. On the selection of the experimental animals extensive calculations were devoted to this aspect. The weight proportions between the smallest and largest experimental animals, on which the measurements were based, lay between 1:22 and 1:50 (cf. Figs. 2–6). Very young specimens up to a body length of 4–5 mm. could not be considered, since on account of their minute size they escaped from the respiratory chamber into the capillary system of the flow-through apparatus, and could thus disturb the measuring process. Besides, it appeared prudent to dispense with groups of this size on account also of the very low flow-through speeds which were necessary for experiments on small animals of this kind, and on account of the greater inaccuracy of the measurements connected with this.

Since for intra – and interspecific comparisons, it appeared better to work from a greater difference in the salinities to be compared, the measurements were made in two concentrations, 10^{o}/oo and 30^{o}/oo. Both salt content degrees comprise a region in which these five species also occur naturally, although in part only temporarily. At least with the 30^{o}/oo stage the approximate upper limit of the natural distribution of G. duebeni and G. zaddachi is attained. Later the particulars of the salt content requirements of these species will be examined in detail.

The respiratory measurements, which were continuously made on long-term adapted individual animals, lasted as a rule for several hours, until constant metabolic values were obtained. By means of the course of the respiratory curves, it could be estimated when approximately basic metabolic conditions were reached. At any rate it can be stated, with reservations, that for the experimental animals a full rest position was practically never given. Unless measurements were undertaken on drugged shrimps, as FOX & SIMMONDS (1933) as well as LÖWENSTEIN (1935) did, when definite effects of the narcotic on the metabolism could certainly not be precluded, definite spontaneous activity must be tolerated. This also explains the not inconsiderable range of variation of the measured data, which are reproduced in Figs. 2-6. In these graphical representations, regression lines calculated for both salinities have been inserted. For the regression analysis the oxygen consumption values for male and female as well as for young animals not yet sexually differentiated were included, since no specifically sexual differences could be proved. The allometric parameters a and b with their standard deviations s_a and s_b calculated out of all the measured data, are placed in Tab. 1 next to the standard estimated error $s_{y.x}$ as the standard of the dispersal around the regression line and the correlation coefficient r. These facts permit the corresponding confidence intervals for a selected confidence probability to be determined (cf. SACHS 1972), and other statistical calculations to be conducted.

If one should compare the regression coefficients on an intra- and interspecific level, it becomes plain that in the 30°/oo stage higher values than in the 10°/oo stage are to be recorded for all species, with the exception of G. duebeni. This means that the slope of the regression line in the higher salinity is steeper for G. locusta, G. oceanicus, G. salinus and G. zaddachi than in the lower concentration stage, while for G. duebeni almost identical proportions are present in relation to the slope of the line in both series of experiments. With an intraspecifically adjusted statistical comparison of the regression coefficients between the respective 30°/oo and 10°/oo experimental ranges, for all five species highly significant differences with an error probability of 0.1% are given.

It is hence remarkable, that there is present a considerable variability in the regression coefficients. These range from values of 1 for G. locusta in 30°/oo up to values of 0.6, which have been computed in 10°/oo for G. salinus and G. zaddachi, and for G. duebeni in both salt content regions. From these results it is clear that the metabolic size ranges from a weight proportionality to a surface proportionality; in some cases even a relation underlying a surface proportionality can be established. Such low regression coefficients are also known in isolated cases of other crustaceans, as emerges from a compilation by WOLVEKAMP & WATERMAN (1960, p. 45). As the experimental animals included fairly wide ranges in weight, a sufficiently accurate determination of the regression coefficients should be guaranteed.

If one considers the amount of the oxygen consumption next on the basis of an interspecific comparison, it emerges that in most cases no positive relations can be deduced, without taking the body weight into consideration. Since the regression lines show more or less different slopes, the differences between both salt content stages are dependent on the size ratios in connection with the average metabolic rate (Figs. 2-6)*. Thus for G. locusta and G. oceanicus a higher average oxygen need is recorded in the area of weight below 0.06 g. or 0.2 g. respectively in 10°/oo than in 30°/oo, where the differences with decreasing size are increasingly more marked. As the regression lines of G. locusta are cut off at a body weight of about 0.06 g., this ratio begins to become inverted above this weight stage. With G. oceanicus meanwhile the intersection of the line lies almost outside the weight area being examined. The other three species exhibit much smaller dependence of respiratory intensity on salt content. With G. salinus and G. zaddachi, the differences increase with body weight, where the oxygen need in the salt-rich stage is somewhat higher than in the poorer salt medium.

If one obtains the oxygen consumption at a living weight of 0.1 g. there results from an interspecific comparison ranging from G. duebeni through G. zaddachi, G. salinus, G. oceanicus to G. locusta, a tendency to an increasingly higher respiratory intensity in both salt content stages. G. zaddachi and G. salinus show the smallest differences in relation to their metabolic size. G. locusta however is clearly contrasted with the other species through a relatively high oxygen requirements, where the ratio of the average Q_{O2} between G. duebeni and G. locusta amounts to more than 1:2. As can be calculated from the data in Tab. 1, this ranges from 7.1 to 17.1 cu.mm.^{3} 02/0.1 g/h (30°/oo) and from 7.4 to 15.4 cu.mm.^{3} 02/0.1g/h (10°/oo) respectively.

Finally, some indication should yet be made of the relatively high values for the correlation coefficients (cf. Tab. 1)*, through which the narrow

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* Figures and tables are at the end of this translation.

relationship between oxygen consumption and body size finds its numerical expression.

To complete the experiments on the dependence of size on respiratory intensity thus far described, a particular parasitological question was incorporated into the metabolic analysis. Here it is a case of a comparison of the respiratory amounts of individuals which were infected with certain microsporidae, and that of uninfected animals.

In the framework of extensive studies on the sexual determination of G. duebeni the occurrence of two microsporidae species, Octosporea effeminans and Thelohania herediteria, was established; these exercise a sex determinative influence. They are transferred via the eggs onto the offspring and have the effect of causing all young infected animals to develop into females, also exceptionally into intersexes (cf. BULNHEIM 1969, 1972). Both species colonise the ovary, Thelohania herediteria in addition parasitizes ~~in~~ the entire bodily musculature. In order to be able to state whether the respiratory metabolism is also affected through infection with microsporidae, parallel measurements were carried out between uninfected and Thelohania-infected individuals of G. duebeni which - as all other experimental animals - also derived from laboratory cultures. The results of these experiments are shown in Tab. 1 as well as Fig. 6.

A significance testing of the regression coefficients between uninfected and infected females including young animals not yet differentiated sexually gave no definite differences at an error probablility of greater than 2%. The respiratory intensity of the shrimps experiences no variations in weight in spite of the quite severe parasitism, which underlines the generally negligible pathogenic effect of these microsporidae (BULNHEIM 1971).

It becomes further clear, from the comparison of values which scarcely differ from each other of the allometric parameters for the females and young animals on the one hand, as well as for individuals of both sexes on the other hand (Tab. 1), that no sexually specific differences are present.

Effect of an abrupt change in the salt content

Organisms which colonize brackish-water biotopes are exposed to strongly changing conditions of salinity. In river mouth regions, in the transition zones between freshwater and marine environments, there occur particularly drastic fluctuations of salt content, which can be of short-term (tidal) as well as long-term (seasonal) nature. Limited through these and other constantly changing environmental factors, estuary dwellers are particularly strongly occupied with their physiological and ecological reactions. They are thus provided with certain compensatory abilities, such as for example pronounced osmo-regulatory abilities, in order to be able to resist the changing outer conditions.

As the Gammarus species under investigation also inhabit estuaries for the most part, and are exposed to a constant osmotic stress through the variations in salt content which prevail there, tests were made on how a sudden change of the salinity affected the respiratory intensity in connection with the problem of the dependence on size of respiratory metabolism. In connection with the osmo-regulatory processes connected with this, the question of overriding interest concerned the interval of time for environmental adaptation, the replacement of the metabolic position from the original level to a new position of equilibrium. Thus these experiments followed the object of throwing light on the extent of the physiological adaptability to salinity alterations from the point of view of an interspecific comparison.

As experiments by BEADLE & CRAGG (1940), KINNE (1952) and WERNTZ (1963) have shown, Gammaridae are classed as hyperosmotic regulators. Marine species are able to regulate the osmotic concentration of the blood more strongly than brackish-water forms, which freshwater species possess the most negligible regulatory ability. This ability is vouched for by the fact that the former are in a position to maintain a definite osmotic concentration gradient between inner and outer media over much larger areas of salinity than the latter.

While carrying out experiments on the effect of sudden alteration of the salt content, it was worth considering the salinity tolerances of the individual species. The lower tolerance limits, which restrict natural occurrence in brackish waters, show distinct differences for the <u>Gammarus</u> species under investigation, as can be concluded from numerous ecological data on their distribution. <u>G. locusta</u> tolerates salinities of 5-6°/oo in the Baltic, while the presence of <u>G. oceanicus</u>, as well as of <u>G. salinus</u>, is limited by salinities of 2-3°/oo (SEGERSTRÅLE 1947, 1959). All three species, however, also occur in the pure marine region. The presence of <u>G. zaddachi</u> was established in salinities of between 0.1 and 33.4°/oo in the neighbourhood of a river mouth system (STOCK et al. 1966). <u>G. duebeni</u> is, like <u>G. zaddachi</u>, a typical brackish-water form. They occur preferably in meso- and oligohaline waters; both species are even able to penetrate into freshwater biotopes (cf. inter alia HYNES 1954, SUTCLIFFE 1967, PINKSTER et al. 1970). In addition, <u>G. duebeni</u> has also been found in waters with a salt content of up to 30°/oo, and even isolated specimens in yet higher concentrations (45°/oo) (FORSMAN 1951).

Arising from the euhaline and mesohaline salinity area respectively and the change from one stage to the other, the abrupt transition to oligohaline and polyhaline stages also belonged to the experimental programme. In order not to subject the shrimps to too severe a physiological stress, the difference of the individual salt content concentrations to which they were exposed amounted at the most to 20°/oo. In individual cases the following series of tests were carried out: abrupt change from 10°/oo to 30°/oo and reverse, from 30°/oo to 45°/oo as well as from 10°/oo to 3°/oo.

All measurements were made on adult individuals, which had experienced long-term adaptation to 10°/oo or 30°/oo under constant laboratory conditions. Next, respiratory measurements extending over several hours (more than 8) were made in these salt content stages. Only then there followed an abrupt change in salinity with continuous recording. As it was clear that in general critical alterations in the metabolic intensity were taking place within one day, although mostly directly after the salt content change, the measurements were not as a rule protracted beyond 35-40 hours. Figs. 7 to 23 illustrate what alterations the respiratory metabolism has experienced in the different test series.

As was brought out in the preceding section, no proportionality exists between body size and respiratory intensity - apart from G. locusta in the 30°/oo stage - except for a more or less superficial relationship. On this basis there remained a connection of the metabolic values with a definite unit of weight. As experimental animals of equal weight were not further available for all species and all series of tests, there followed a conversion of the measurement data into relative values, which moreover permitted a better comparison of the results. This took place according to the following principle: the respiratory sizes obtained in the first salt content stage served as relative values for all variations measured later. These data (oxygen consumption per hour) which had been recorded over a period of 5-6 hours before the change in salinity were made a continuous basis. These were determined for every individual experimental animal and set equally at 100%. Higher metabolic values caused through strong locomotive activity were not taken into consideration. A change in salinity only followed when a constant respiratory intensity could be shown. The data recorded in the second salt content stage were accordingly expressed as relative values likewise, ie. as percent~~ual~~age deviations as opposed to the original values of the first salinity stage. The relative values obtained for each experimental animal per hour were added, and the average values, average error of the average value and standard deviations were calculated. At least 5-6 shrimps were tested on average per series of experiments. As no discrepancies dependent on sex were evident, the data for female and male specimens could be taken together; in many cases the experiments were at any rate carried out exclusively on individuals of one sex. For species which were distinguished through greater differences of the regression coefficients, attention was paid to the fact that individuals subjected to a series of experiments originated from approximately equal weight classes.

During the process of adaptation, which is set off through the influence of sudden alterations in the intensity of certain environmental factors, there may come to light a reaction consisting of three phases (cf. PROSSER

1958, KINNE 1964). In a typical case this begins with a shock reaction lasting for only a short time, which is expressed in the form of an "overshoot". Then follows the stabilising phase, which lasts several minutes, hours or days. This passes finally into the phase of a new, stable condition, at which the adaptation to the altered environment is completed. As Figs. 7-23 illustrate, these individual phases, as far as the variations of the respiratory intensity reflect the process of adaptation to salt content, are contrasted more or less clearly with each other. In some cases, however, the shock reaction and the phase of the new stable condition do not appear so clearly demarcated.

Next are set out the results which were obtained on the transference of the Gammaridae from a dilute to a concentrated medium.

It may generally be established, that at a leap in salinity from 16°/oo to 30°/oo the establishment of a new metabolic level takes place fairly rapidly; after already about 3 to 5 hours almost constant oxygen consumption values are reached (Figs. 7-9). Here there takes place a small decrease in the size classes of G. locusta and G. oceanicus, under examination, a slight increase of the ~~same weight group~~ respiratory intensity in the case of G. zaddachi, while for G. salinus and G. duebeni (individuals of the same weight group) no further variations are shown. As the curve for G. locusta and G. oceanicus on the one hand and for G. duebeni and G. salinus on the other seemed quite similar, the results in each case are depicted for only one representative of this species.

The more or less strong slope of the respiratory metabolism, which in this and in the other series of experiments can appear directly after the change in salinity is attributable partly to an increase of the locomotive activity. ~~At first in many~~ With the alteration in concentration in the outer medium at first in many cases there is a flight reaction, which is reflected in strong motor activity. Such phases of activity, which in general are only short-term, can also occur further on during the process of adaptation. They were not, however, considered in the evaluation. This explains why the average values given per hour are not uniformly related to the same number of experimental animals.

The not inconsiderable range of scattering of the measurements is attributable in the first place to individual differences, which come to light in relation to the reactions of the experimental animals, also however to a lesser extent to a somewhat heightened spontaneous activity, which however generally dies rapidly away. It should hence be stressed that in view of the marked difference in the curve from species to species there is no occasion for linking the variations in respiratory metabolism observed, with a heightened locomotive activity in general.

The next series of experiments dealt with an abrupt change from 30°/oo to 45°/oo. While for G. locusta there appeared a somewhat diminished oxygen requirement, in the case of G. oceanicus and G. salinus there was a stronger rise in metabolism, and a smaller one for G. duebeni (Figs. 10-13). After about 4-7 hours almost constant average values were attained. Thus the process of adaptation takes place fairly swiftly. The reactions of G. zaddachi were not examined, since the tolerance of this species in relation to polyhaline media is relatively poor. The other species better withstood a transition of 30°/oo to 45°/oo, as some supplementary control series showed. And so the mortality in my medium of 45°/oo lay at between 20% and 30% within a period of 12 days for G. locusta, G. oceanicus and G. salinus; this amounted to only 5% for G. duebeni.

Two further series of experiments were finally of value to the question of the nature of the metabolic reactions and their duration at a change from higher to lower salinities. The plunge in salt content from 30°/oo to 10°/oo as a postscript to the first test series afforded quite another picture (Figs. 14-19). Apart from G. duebeni with a generally constant metabolic level, which is only interrupted by an overshoot reaction, the respiratory intensity for the other species undergoes a more or less drastic increase. This is greatest for G. locusta with an initial increase of c. 70% above the original values, Fig. 19 also illustrates the marked difference in the method of reaction of both species, which the result of a repeated change reflects. For the other three species, especially G. zaddachi, the respiratory gradient does not show such a considerable amount. The variations of the QO_2 during the stabilisation phase show a much longer course than with the transitions from a lower to a higher degree of salt content. Only after about 20-30 hours is an almost new "steady state" attained, at which G. locusta and G. oceanicus maintain, as expected, a somewhat higher level than in the first salt content stage. This is also the case with G. salinus; the final values which were observed for the range of size being examined, lay on average a little higher than with the individuals adapted to 10°/oo on the long-term. Although also in view of the relatively small number of individual measurements we are dealing with a result dependent on chance, these facts suggest that the final adaptation to a new stable condition for G. salinus within the observation period could not yet have possibly taken place fully. This fact should also hold true for G. locusta. Some measurements, which ranged up to 48 hours after the plunge in salinity, showed that the oxygen consumption could decline yet further; at any rate not inconsiderable individual differences were outlined here.

It should in addition be noted that not all individuals survived the sudden transition from 30°/oo to 10°/oo. Here substantially more measurements were made on G. locusta than for the other species. At the evaluation in general only those animals which had survived the salt content change without detectable damage were taken into consideration.

The leap in salinity from 10°/oo to 3°/oo represents indeed only a small difference of concentration, but this lower degree of salt content already marks the limit of tolerance for G. oceanicus and G. salinus. As it lies higher for G. locusta, corresponding experiments on this species were not undertaken.

As exemplified in Figs. 20-23, in the case of G. oceanicus there occurs a strong increase in oxygen requirement directly after the change, and this then shows a tendency to go in reverse, and reaches equilibrium after about 15 hours at a level which remains constant. G. salinus reaches a new constant value with a longer slope after about 7 hours. In contrast to the original situation the oxygen consumption in this new stable phase still rises by about 30% (G. oceanicus) and 20% (G. salinus) respectively. Likewise with G. zaddachi there occurs an increase with a following decrease, which is apparently over after 20-25 hours. Similar behaviour is also shown for G. duebeni, although the metabolic increase is substantially less and the original values are almost attained again in the final stage of the stabilisation phase.

In conclusion it may be established that in general the process of adaptation after abrupt alterations in the salt content proceeds substantially more slowly, and is linked with strong alterations in the respiratory intensity, if the change from concentrated to dilute media is made, than by transition in the opposite direction. The question as to how far these metabolic reactions are connected with the osmo-regulatory processes taking place here, will have to be discussed later.

Effect of a long period of starvation

That the metabolic intensity of poikilothermic animals declines when they are deprived of food is a fact borne out by numerous experiments. Less well-known is the period of time for this reduction in metabolism. Experiments on the effect of a long-lasting fast should thus clarify to what extent the oxygen consumption declines during a longer period of time. From these experiments information was at the same time expected on how far the metabolic level could be additionally altered on the basis of a condition of starvation, in experiments on the effect of an abrupt change in the salt content, extending likewise over a longer period.

Fig. 24 illustrates the variations in respiratory intensity of Gammarus oceanicus at being deprived of food for 6 days. On account of considerable initial irregularities, the evaluation of the measurements began only four hours after the experimental animals had been placed in the respiration chamber. The shrimps had been previously provided with sufficient food. As emerges from the curve, a relatively strong reduction in metabolism is recorded within the first 12 hours. After about 20-24 hours approximately constant values are attained, lying about 15% below the original level; later on also these diminish in only very small amounts.

Parallel measurements made under the effect of a longer condition of starvation on two examples of G. duebeni, which ranged over one week, gave no picture diverging from the ratios for G. oceanicus, so that calculations could also be made for the other species in a similar situation with regard to the reduction in metabolism during starvation.

Effect of activity and the ventilation rhythm

As with many animals which dwell in the tidal zone, amphipods are also known to have periodicity phenomena, which are underlain by internal rhythms. For different species - also for Gammarus species - there was shown an activity rhythm connected with the day- and night-cycle and the tide cycle, on which numerous monographs are available. JANSSON & KALLENDER (1968) observed heightened nocturnal movement for G. oceanicus as well as DENNERT et al. (1969) on G. zaddachi. HALCROW & BOYD (1967) established in addition for G. oceanicus a period of activity correlated with the tidal phases as well as FINCHAM (1972) for Marinogammarus marinus. HALCROW & BOYD however recorded a rapid decline of this rhythm under laboratory conditions where there was no time indication.

An analysis of possible effects of internal rhythms on the metabolic level was not made in the immediate centre of interest of the present experiments. As had already been stated, the experimental animals originated almost without exception from laboratory cultures; they thus offered no assurance that there persisted periodicity phenomena occurring in the wild. Thus a clear working of these effects could not be recognised - especially the long-term measurements over several days on the determination of the metabolic reduction under conditions of starvation. Indeed, in some cases there was recorded a definite nocturnal increase in metabolism, provoked through heightened locomotive activity, but for the most part there emerged no daily fluctuation in oxygen consumption under the conditions of a natural light-dark change, such as prevailed in the laboratory during the measurements. Some steering experiments on specimens of G. duebeni, which had for a long time been exposed to a light-dark change for 12:12 hours before and also during the measurements, likewise did not allow the emergence of metabolic rhythm to be clearly recognised.

The state of activity of the experimental animals could be judged quite accurately be means of continuously recorded measurement values. Through observation of their behaviour in the respiration chamber on one hand and comparison of the recorded curve on the other, a clear picture could be obtained on whether the shrimps remained in a state of rest or made movements. Here the metabolisms for rest and activity could be distinguished with greater certainty, and placed in relation to each other. Thus in the case of adult individuals in a state of active movement, these could be measured a rise of the metabolic level ~~of~~ up to 2.5 to 3 times compared to the rest values; for specimens of <u>G. duebeni</u> there was an even more than threefold rise in oxygen need. Here it should be taken into account that the small size of the respiration chamber permitted no continuous swimming movements, only a waving motion backwards and forwards, as well as rotation on the body axis. The values given here agree quite well with the data of HALCROW & BOYD (1967) in relation to size. These authors carried out combined respiratory and activity measurements on <u>G. oceanicus</u>, depending on the temperature, and at 15°C determined rises in oxygen consumption during phases of swimming of around 2.5 times compared with the rest condition.

Mention has already been made that no complete state of rest was given for the shrimps in the respiration chamber over a prolonged time, but a definite spontaneous activity must be taken into account, being expressed in occasional movements of the body and the extremities as well as the ventilation activity of the pleopods.

Through the beats of the pleopods directed towards the tail, a stream of water is driven from the front to the rear end, ensuring a constant renewal of the respiratory medium along the surface of the gills and therefore a maximum concentration gradient for the exchange of gas. On the question of the regulatory mechanism of the respiratory movements, experiments on the dependence of the respiratory activity of the pleopoda on oxygen pressure have been carried out for <u>G. pulex</u>, <u>G. locusta</u> (WALSHE-MAETZ 1956) and <u>Marinogammarus obtusatus</u> (GAMBLE 1970) as well as on other amphipoda. These showed that a reduction of the oxygen concentration of up to c. 20% of the normal value leads to a marked increase of the frequency of beating, while yet smaller concentrations effect a decrease of ventilation activity. With oxygen saturation of the respiratory medium, GAMBLE observed almost complete continuous activity of the pleopods, which was however more or less irregular.

For all the Gammarids investigated here, there is also mostly no movement regularly following the beating of the pleopods when at rest. After the experimental animals had a long and undisturbed stay in the respiration chamber under basic metabolic conditions this respiratory ventilation mechanism can however assume a more or less pronounced rhythm. As the recorded examples in Fig. 25 make clear, such periodical ventilation movements are reflected in the quite regular variations of the respiratory curve. In more or less rhythmical motion sequences, phases of the pleopod beating then alternate with phases of the pleopod inactivity, so that the curve takes on an indented appearance. During the ventilation movements the oxygen consumption increases, to fall again at the moment the pleopods come to rest. The amplitude of the variation, as was shown on the compensation writer, was shown to be dependent on the amount of water passing through, and on the size of the individual. As far as the ventilation rhythm is modified through further factors, as for example temperature, was not investigated within the content of this work. The rate of water-flow, which was tested in the region between 12 and 60 ml/h., influences the amplitude in that its height decreases with increasing volume of flow, without causing a change in the frequency of the alternating active and rest phases of the pleopods. The difference of the oxygen pressure, which was attained between the beginning and end of the beating activity, amounted in general to not more than 8 Torr for even a small flow of water, and as rule there ensued much smaller values. It should however be stressed that these facts have as a prerequisite only a small respiratory decrease in the oxygen pressure, which under the given experimental conditions in general amounted to less than 10% of the saturated value.

The influence of the individual size on the size of the amplitude is shown in that this becomes smaller with decreasing body size. Moreover, the respiratory curves for animals not yet sexually mature show in general a less pronounced rhythmical sequence of variation. The height of amplitude also presents no unalterable size with a state of complete rest. It may become larger or smaller in the course of longer periods of time. Should the latter be the case, then the frequencies of the active and rest phases respectively of the pleopods increase.

The previously described regularities of the curve could only be recorded for isolated individuals of G. salinus, G. oceanicus and G. duebeni; for G. zaddachi they did not appear in so pronounced a form, and also for G. locusta the form of the curve in general revealed no proportional sequence of variation.

If one compares the recorded examples in Fig. 25, it becomes clear that the highest number of alternating active and rest periods per unit of time is recorded for G. salinus; it is at the lowest for G. duebeni, while G. oceanicus occupies a middle position. In the example reproduced here for G. duebeni the period length of an active phase amounts to about 8-10 minutes up to the next one; even 20-30 minutes were attained as extreme values for this species, at which the rest phase of the pleopods increasingly lengthened.

No quantitative data have been obtained on the number of beats of pleopods during the phases of activity. By means of the curve and on the basis of visual observations it can however be established that the number of movements of the pleopods per unit of time is relatively lower for G. duebeni than for G. salinus and G. oceanicus.

From the examples demonstrated, and others not documented here, one can nevertheless infer differences in the ventilation rhythm which are connected with the species only in a very limited manner, especially the form of the curve presented - apart from numerous individual discrepancies - appear only after prolonged starvation, which is connected with a cessation of locomotive activity. On the other hand the relatively small frequency of alternating movement and rest phases of the pleopods recorded for G. duebeni can be evaluated as a characteristic typical of the species, one which can be linked to the proportionately lower oxygen requirement of this species.

Effect of exuviation

The process of growth in crustaceans takes place in stages of moulting, which are governed by a hormonal disturbance as well as the influence of different outside factors. The moulting cycle is linked with numerous changes of basic, interacting metabolic processes, which affect amongst others the conservation of water, the metabolism of minerals, the turnover of reserve substances and the respiratory intensity.

The influence of moulting on the respiratory metabolism has hitherto been above all investigated on various decapoda, for which there has generally been recorded a rise in oxygen requirement during the casting-off of the old exoskeleton (e.g. SCUDAMORE 1947, FROST et al. 1951, SCHEER & SCHEER 1954, ROBERTS 1957, HEINEMANN 1964). Corresponding data are available for the shrimp (? Leuchtkrebs) *Euphasia pacifica* (PARANJAPE 1967) and the wood-louse *Porcellio scaber* (WIESER 1965). With *Porcellio scaber* the rise in respiratory metabolism before moulting begins with the cessation of the food intake . The oxygen requirement reaches two steep maxima; the first maximum coincides with the ecdysis posterioris, the second with the ecdysis anterioris. COSTLOW & BOOKHOUT (1958) found no increase in breathing for the cirripede *Balanus improvisus* var. *denticulata*, while BARNES & BARNES (1963) on the other hand were able to show a small increase in oxygen consumption during moulting for *Balanus balanoides*. No data are available for the relevant ratios for Gammaridae - apart from an experiment on *G. duebeni* on the transport of sodium before and after moulting (LOCKWOOD & ANDREWS 1969).

In the framework of the given possibilities of measurement, the variations of oxygen intake, which take place during the process of moulting, could be continually shown for all five *Gammarus* species under investigation. In all ten measurements were evaluated on animals of both sexes.

As was shown to agree for all five species, with Gammaridae also a general increase in respiratory intensity precedes the process of separation of the old cuticula from the epidermis. The respiratory curves on average reach a summit 5-12 hours after the beginning of the rise, which coincides with the actual act of moulting (Figs. 26, 27). The casting-off of the old exoskeleton takes place very rapidly; this process lasts not longer than about one minute. The casting of the exuviae is accompanied by strong motile activity. To what extent the increased consumption of energy is to be explained through the rise in movement, and how far in addition other metabolic processes accompanying the process of moulting, such as the active intake of water, condition the heightened oxygen requirement, cannot be clearly judged. The rise in breathing before moulting is indeed always connected with an increase in activity, though by means of the curve the intensity with which the shrimps move can be estimated. It can however be concluded from available recordings that the increase in metabolic performance can be attributed only in part to a rise in activity.

The increase in metabolism which is attained at the moment of casting off the exoskeleton, lies between the 2.2 and 3.9 of the normal level attained before and after moulting respectively. The smallest increase could be recorded in an example of <u>G. duebeni</u> and the highest in individuals of <u>G. locusta</u> and <u>G. zaddachi</u> (Tab. 2). After the exuviae are cast away, the oxygen consumption next falls rapidly, then, about 1-2 hours later, somewhat more slowly. In many cases the decrease of the respiratory curve shows a less steep slope than the increase before moulting, so that there are given unsymmetrical forms of the curves. In the course of 8-20 hours, at the most 30 hours after moulting, the original values for oxygen consumption are again attained.

The duration of the alterations in metabolism takes place in approximately the same way for the individual species, although a definite width of variation can be established for the values determined. This makes recognisable a more or less clear dependence on the size of the individual. The variations in respiratory intensity in general level out to a normal value faster in smaller animals than in large ones. Obviously this state of affairs has a causal relationship with the moulting frequency, which under constant environmental conditions is higher for young individuals than for adults. As the intervals between moulting become increasingly longer in the course of growth (with the exception of females during the reproductory stage) these changes in metabolism also experience a corresponding lengthening of time.

As emerges from Tab. 2, no pronounced interspecific differences can be identified in view of the width of variation of the data for the maximum oxygen consumption during the casting-off of the exoskeleton. The lowest metabolic values were determined for G. duebeni and the highest for G. locusta. These findings correspond to the different oxygen requirements of the individual species, which - as previously specified - show the smallest values for G. duebeni and the highest for G. locusta.

DISCUSSION

The following considerations are of value in the discussion of some metabolic~~o~~-physiological questions, which result from the findings of the present work. Finally the results of the respiratory measurements - as far as these have attained significance for a causal understanding of the environmental relationships of the Gammarus species under investigation - are discussed from ecological viewpoints.

Physiological aspects

The experiments on the dependence on size of the respiratory metabolism dependent on the salinity of the exterior medium have shown that - apart from Gammarus locusta in the 30^{o}/oo stage - with increasing body weight there appears a relative decline in the intensity of metabolism; The reduction in metabolism in the course of growth is characterised through values, which lie between weight and surface proportionality, or even below these. As the measurements in both salt content stages being examined have shown, the regression coefficients for each species are identified through significantly different values.

The dependence of this coefficient on the salinity of the exterior medium has also been established, for example, for Artemia salina (GILCHRIST 1956), Metapenaeus monoceros (RAO 1958), for Hemigrapsus species (DEHNEL 1960) and Corophium volutator (McLUSKY 1969). The data provided afford further evidence on the not inconsiderable variability of the regression coefficient and its control through the salt concentration of the exterior medium.

Numerous deliberations have been made in metabolico-physiological literature on the significance of the size of the regression coefficient. LEHMANN (1956) considered the regression coefficient to be a constant, typical of the species, established by heredity, while VON BERTALANFFY (1957) placed this in relation to definite systematic categories. HEMMINGSEN (1960) came to the conclusion that an exponent of 0.75 is of general value for poikilothermic animals. Meanwhile, however, numerous data have been recorded which have refuted and disproved these conclusions. Not only does the factor of salinity affect the size of this coefficient, but it is also dependent on numerous other variables, eg. the state of activity (WESEMEIER 1960), temperature (KRUGER 1964, BARNES & BARNES 1969), infection through parasites (DUERR 1967) and the nature of the respiratory medium (NEWELL et al. 1972). Annual and population-conditional differences in metabolism (FISH & PREECE 1970) may also be reflected in the size of the regression coefficient. The origins for this inconsistancy have hitherto remained unclear. Without deepening the discussion of the problem which is presented at this point, KRUGER's view (1964) should be agreed with, that the considerable variability of the regression coefficient, which is here proved only through a few examples, divests the latter of a basic size in every relationship of its character.

Different aspects of the respiratory metabolism have already been investigated in several representatives of the genus <u>Gammarus</u> (SCHAPERCLAUS 1925, SCHLIEPER 1931, FOX & SIMMONDS 1933, LOWENSTEIN 1935, KINNE 1952, TROIANI 1954, KROG 1954, SUOMALAINEN 1958, LUKACSOVICS 1958, WAUTIER & TROIANI 1960, WOYNAROVITCH 1961, ALI & STEELE 1962, HALCROW & BOYD 1967, ROUX & ROUX 1967, CULVER & POULSON 1971, ROUX 1972). By means of numerous data given in the literature, an experiment was undertaken to compare the amounts of oxygen consumption for the individual species, in order to contrast the related values with the five species under investigation in this work. Here it became clear that a comparison with other data on the amount of metabolism in the individual <u>Gammarus</u> species is only possible conditionally. The main reason for this restrictive finding is that almost without exception only data on the respiratory size per unit of weight are available. On the basis of the reduction in metabolism during the course of growth, which should prevail according to the experimental results obtained for Gammaridae, this form of giving the intensity of metabolism is inadequate. The determination of the allometric parameters on the characterisation of the relationships between respiratory metabolism and body weight should rather be regarded as a prerequisite for interspecific comparisons. Such an intention is moreover burdened with further uncertainties, which consist, inter alia, of the fact that different methods of investigation lie behind the individual works, no uniformity of the related size is realisable for the oxygen consumption, and in addition false diagnoses on the species under examination cannot be excluded in some older publications.

On the above-mentioned shortcoming, the statement of intensity of metabolism per unit of weight without regard for dependence on size, the discrepancies of the individual results should particularly be referred to the data of SUOMALAINEN (1958) which in addition are not based on individual measurements. He recorded quite high metabolic values for G. duebeni and G. oceanicus at 15°C and 10°/oo, while for G. zaddachi in contrast with these species he determined an oxygen requirement almost half as high again. For G. oceanicus SUOMALAINEN found a raised QO_2 in 7°/oo and 4°/oo, and the same for G. zaddachi in 4°/oo . In 1°/oo he established a strong decrease for G. oceanicus, and a weak one for G. zaddachi, while he recorded no substantial alterations in respiratory intensity for G. duebeni in the region between 20°/oo and 1°/oo. KINNE (1952) on the other hand found a relative increase in oxygen consumption for male specimens of G. duebeni in 5°/oo and a strong rise in the region of 2°/oo to fresh water, while he recorded a maximum oxygen consumption of 5°/oo, with a weak decrease in 2°/oo and in fresh water for females, for which on the whole higher values were obtained. In salt contents of over 22°/oo and 30°/oo respectively he found for females and males a negligible decrease of the QO_2. The sexually specific differences which KINNE observed are obviously attributable to the fact that the females which were measured bore eggs of young in the marsupium almost without exception. This state of affairs
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should have occasioned an influence on the measured values. KROG (1954) on the other hand found no sexually-based differences for *G. limnaeus*, a result which also corresponds to my own findings.

As far as the data on record allow an interspecific comparison amongst the provisos dealt with above, the conclusion must be drawn that freshwater *Gammarus* species show a higher metabolic intensity than euryhaline forms. This is supported by the comparative experiments of FOX & SIMMONDS (1933) on *G. locusta*, *G. marina* and *G. pulex* as well as by SUOMALAINEN (1958) on *G. duebeni* etc., which in each case were treated with the same method. This finding is emphasised by contrasting the relatively high respiratory values - related to the dry weight and a temperature of 15°C - for the freshwater forms *G. pulex*, *G. fossarum* (1200 cu.mm.^{3} O_2/g/h) and *G. lacustris* (1050-1300 cu.mm.^{3} O_2/g/h) (ROUX & ROUX 1967, ROUX 1972) with the data for the euryhaline species under investigation here. In relation to the dry weight, there only arose for *G. locusta* values (900 cu.mm.^{3} O_2/g/h at 30^o/oo) which lay only a little below the size of the freshwater gammaridae, while the data for the euryhaline species *Marinogammarus (Gammarus) marinus* and *G. chevreuxi* (FOX & SIMMONDS 1933, LÖWENSTEIN 1935) seemed to correspond more to the oxygen requirements of *G. salinus* and *G. oceanicus*.

The alterations in metabolism after a change in salinity are closely linked with osmotic and ion-regulating processes, which lead to the establishment of a new level of concentration of extra- and intracellular body liquids. In the following some aspects of the osmotic adaptation for shrimps and their relationship with the respiratory metabolism are discussed.

For all *Gammarus* species hitherto investigated, in which we are dealing with sea and brackish-water dwellers as well as freshwater ones, the property of hyperosmotic regulation is common, although depending on the relation to different environments there exist differences with regard to the osmo-regulatory capacity. Amongst the species treated in the present work, only the extracellular anisosmotic regulation of *G. locusta*, *G. duebeni* and *G. oceanicus* has been examined (cf. BEADLE & CRAGG 1940, KINNE 1952, WERNTZ 1963).

The interior osmotic concentration is shown through an increasingly hypertonic reaction at below 30°/oo for *G. oceanicus* (WERNTZ 1963), 24‰ for *G. locusta* (BEADLE & CRAGG 1940) and 22°/oo for *G. duebeni* (KINNE 1952). With progressive dilution of the exterior medium the osmotic concentration of the blood decreases only by a small amount, whereby the osmo-regulatory efficiency rises constantly in relation to the difference in concentration between internal and external media. For *G. duebeni* and *G. oceanicus* it attains a maximum in oligohaline stages (<5°/oo) and falls rapidly in the lower limit region of salinity tolerance, in which there is finally a complete breakdown of the regulatory mechanisms. Above the brackish-water stages mentioned and in sea water a poikilo-osmotic behaviour exists. In this area there are almost isosmotic conditions, although there survives a weakly hypertonic reaction of the bloodstream. It is to be taken that the regulation of the osmotic concentration of the haemolymph for *G. salinus* and *G. zaddachi* follows in a similar fashion.

Within the framework of the present experiments, the question on what rate the osmo-regulatory processes take place on extra- and intracellular level after an abrupt change in salt content, takes on particular interest. Findings on crustaceans, particularly decapods, are available which afford some information on this question of the duration of osmotic adaptation.

GROSS (1957) found that the osmolarity of the haemolymphs for *Pachygrapsus crassipes* attains a new, constant level after an osmotic stress in hypertonic as well as in hypotonic media within 24 hours (at 16°C). Studies by DEHNEL (1962) on *Hemigrapsus oregonensis* and *H. nudus* showed that a new "steady state" of osmotic concentration in the haemolymph sets in within 24-48 hours (at 15°C) after a transition to different stages of salt content. The strongest variations in osmotic values follow within the first three hours. The osmotic adaptation takes place slowest after a change into hyperhaline and oligohaline concentration stages. SEGAL & BURBANCK (1963) arrived at similar results on the isopod *Cyathura polita*, which like the *Hemigrapsus* species, shows a hyperosmotic regulation in brackish-water media. When transferred from 8°/oo to 1°/oo, 16°/oo or 24°/oo, after 3 hours almost constant values of the osmolarity of the haemolymph were attained (at 22°C). If there followed a change to higher degrees of salinity (up to 48°/oo), the osmotic balance was completed only after 24-48 hours.

In an experiment on kinetic aspects of the extracellular anisosmotic and intracellular isosmotic regulation for Carcinus maenas, SIEBERS et al. (1972) found that the oxygen consumption decreases following a plunge in salinity from 11°/oo to 38°/oo and attains a new constant value within 8-12 hours (at 11°C). The concentration of free amino-acids and proteins in the serum falls likewise within half a day to a level which then remains constant. The osmolarity of the haemolymph as well as the concentrations of Na, K, Ca and Mg on the other hand undergo a slow alteration in time; the rise in concentration is completed only after 24 hours. Also the intracellular regulation is completed within one day; only low-molecule neutral sugars attain a new, and indeed considerably heightened level of concentration only after an adaptation period of about 10 days.

Experiments on the adaptability of Carcinus maenas from the Baltic and the North Sea in 15°/oo and 30°/oo showed that the metabolic adaptation of gill tissue is completed only after about 5 days (THEEDE 1964). These, as also further results from KING (1965) show that isolated tissue can behave differently to the complete animal after an osmotic stress.

With Crangon crangon WEBER & SPAARGAREN (1970) found that the osmotic concentration of the haemolymph attains a new stationary value after 15 hours at a change from 30°/oo to 15°/oo (at 15°C). Investigation of the isosmotic intracellular regulation on the same subject gave a rise in ninhydrin-positive substances in the musculature after a transfer from 17°/oo to 33°/oo (at 5° and 15°C), at which a new and constant level of concentration was attained after 24 hours. An abrupt change of salinity in the reverse direction required about the same time for the establishment of a new condition of equilibrium (WEBER & VAN MARREWIJK 1972). The ninhydrin-positive substances of the blood present in a small concentration falls off within a few hours after a transition from 17°/oo to 33°/oo; with a plunge from 33°/oo to 17°/oo on the other hand there follows an increase in concentration of these substances, which only attain a new stationary level after 3-5 days with large individual variations.

In the case of *Palaemon serratus* and *Lysmata seticaudata*, it was established by measurements of the entire osmotic concentration and the electrolytic concentration of the haemolymph that after a transition from 39°/oo to 24°/oo (at 4° and 22°C), there follows a very rapid osmotic adaptation, at which almost stable values are already attained after about one hour (SPAARGAREN 1972). For *Lysmata seticaudata* this process lasts only a trifle longer than for *Palaemon serratus*. There is a relative increase of the concentration of non-electrolytes in the haemolymph directly after the change. While *Lysmata seticaudata* reacts with strong osmotic conformity, *Crangon crangon* and *Palaemon serratus* show a hyper-regulation in low salinities and a hypo-regulation in higher salinities.

A rapid osmotic adaptaion in relation to the osmolarity of the blood could also be established for the amphipod *Corophium volutator*, a hyper-regulating inhabitant of brackish waters. This is completed within 3 hours after a transition from 3°/oo to 10°/oo (McLUSKY 1969).

That this process should also be concluded for Gammaridae within a few hours, and in any case after about a day, is also to be inferred from some data by WERNTZ (1963). He found that for *G. oceanicus* the osmotic concentration of the haemolymph attains constant values within 12 hours after an abrupt transition from 32°/oo to 3.5°/oo (at 16°-19°C). For *G. fasciatus* this is already the case within 1½ hours after a change from fresh water to brackish water of 21°/oo.

Numerous experiments on the connection between respiratory intensity and salt content of the exterior medium have made it clear that quite different relationships can be present. The following reactions may be distinguished in relation to the amount of oxygen consumption (cf. KINNE 1971): (1) increase in subnormal and/or decrease in supranormal salinities, (2) increase in sub- and supernormal salinities, (3) decrease in sub- and supernormal salinities, and (4) lack of recognisable variations. Both the first groups comprise in essence euryhaline invertebrates, the third is represented by stenohaline forms, while the fourth group is represented through holeuryhaline or extreme euryhaline species. As the Gammarus species under investigation are to be classified in the first two groups on the basis of their metabolic reactions, the following two groups should not be studied in further detail.

Experiments on the crustaceans Carcinus maenas (SCHLIEPER 1929), Uca spp. (GROSS 1957) and Hemigrapsus oregonensis (DEHNEL 1960) yield examples for reactions according to the first type. For the second type there can be included, amongst others: Ocypode albicans (FLEMISTER & FLEMISTER 1951), Palaemonetes varians (LOFTS 1956), Metapenaeus monoceros (RAO 1958) and Crangon vulgaris (HAGERMAN 1970).

According to the foregoing results, a general tendency towards increase in respiratory intensity as a consequence of diminishing salt content in the region of suboptimal degrees of concentration is outlined for euryhaline gammaridae. In this connection reference should also be made to earlier data of SCHLIEPER (1931), who established a decrease in metabolism of 18% after transfer from a medium of 16°/oo into sea-water of 32°/oo for G. locusta, as well as VON LOWENSTEIN (1935), who determined a diminished QO_2 of about 20% for G. chevreuxi in contrast to the values obtained in 32°/oo and 8°/oo. However, without knowledge of the allometric parameters, at least without data on the size of the classes under investigation, these and other data on the alterations of the oxygen consumption have no reliable value, as the previous works on the relationships between respiratory metabolism and body weight depending on salt content, have made clear.

As emerges from the present experiments, the respiratory intensity of the individual Gammarus species is almost generally raised and the salinity adaptation requires a longer period of time, in an active salt intake as opposed to a fall in concentration sets in strongly after a change from a concentrated to a dilute medium, in order to keep the inner medium hyperosmotic. The question as to how the alterations in oxygen requirement arise after a reduction or a rise in the salt content, has still received no definite explanation. SCHLIEPER (1929), who was the first to establish a rise in metabolism for some euryhaline animals as a consequence of a reduced salt concentration in the exterior medium, brought this discovery into relation with the intensified osmo-regulatory performance and the associated rise in energy requirements. This explanation was again applied to the corresponding data on the dependence of the respiratory intensity on salinity (eg. SCHWABE 1933, FLEMISTER & FLEMISTER 1951). It must next count as plausible, in that many euryhaline animals exhibit the least oxygen requirements with hyposmotic regulation in a salt-rich exterior medium, if they live under isosmotic conditions. Such relationships are for example shown for <u>Hemigrapsus oregonensis</u> and <u>H. nudus</u> (DEHNEL 1960), as well as for the river crayfish <u>Astacus astacus</u> (PETERS 1935), which consumes less oxygen in an isosmotic medium of 15°/oo than in fresh water.

As the additional energy requirement, which is necessary for the maintenance of osmotic and ion-regulating processes, must be regarded on the basis of thermodynamical considerations as too small (cf. POTTS & PARRY 1964), to be able to clarify extensively the changes observed in metabolism, SCHLIEPER's interpretation has been queried. Moreover, the considerable rise in metabolism which was measured for different euryhaline invertebrates in brackish-water media, presupposes a very small performance for the contributing ion transport systems. Opposed to this are numerous experimental results on isolated tissues. Also the fact that for many species no measurable metabolic differences come to light in different degrees of salinity, or that a diminishing of the respiratory intensity in strongly fresh media has scarcely been recorded, is the conclusion - at least in these cases - reached by the significance of a rise in energy performance through osmotic and ion-regulating processes. SCHLIEPER (1936) further tried to explain the variations in the rate of metabolism with decreasing salt content by a rise in hydratation of the tissues and through this an increased enzyme activity. KING (1965) showed for various marine and brackish-water species, that this explanation also can claim no general validity.

The idea that a rise in oxygen requirement after an osmotic stress should be especially attributed to a flight reaction and increased motor activity of the experimental animals (GROSS 1957, McFARLAND& PICKENS 1965), cannot serve as a complete explanation because of the arguments previously mentioned. Although the shock reaction directly after an abrupt change in salinity can lead in a short time to increased activity and heightened spontaneous activity respectively, the different results on the variations of the respiratory metabolism for the individual Gammarus species speak against this interpretation.

It is also worth asking how far the dependence of the oxygen concentration on salt content at the same partial oxygen pressure, and the diffusion ratios for the amount of respiratory metabolism altered by this are of significance, although this problem should not be discussed more closely here.

From the foregoing investigations and further experimental results which have not been mentioned, which took place on other animal organisms, it emerges that the relationships between respiratory intensity and salinity have still found no satisfactory interpretation.

For an assessment of the osmo-regulatory energy expenditure, it is also necessary to take into account the efficiency of the cellular tranpost mechanisms, on which FLOREY (1970) has drawn attention. For a clarification of the problem in question, there must be taken into account especially the effectiveness and speed of the ion transport, the permeability of the exterior- and cell membranes for electrolytes and water, as well as the mechanism of the intracellular isosmotic regulation. In this connection, experiments on the ion regulation for Gammaridae throw some light on the question of metabolic energy in dependence on the salt content of the exterior medium. They have led to the realisation that marine species show a greater permeability with regard to salts and water as well as a more intensive urine production than brackish-water and freshwater forms (cf. SUTCLIFFE 1968). In addition, brackish-water species, such as G. duebeni and G. zaddachi, are capable, in lower degrees of salt content, of producing a urine which is hyposmotic to the blood concentration (LOCKWOOD 1961, SUTCLIFFE 1967a, 1968), ~~In addition, brackish-water species, such~~ through which the efficiency of the electrolyte regulation is increased.

Ecological aspects

Finally, the physiological findings of the present work should be considered from ecological points of view, and placed in relation to the environmental claims of the individual Gammarus species. Here an experiment should be undertaken to account for their edological differentiation in metabolic performance, adaptive ability and tolerance, especially compared with the factor of salt content. It should in any case be mentioned in advance, that for the present it is not possible to give a comprehensive eco-physiological characterisation of this group of species, since further functional characteristics and regulative properties must be tested experimentally under comparative viewpoints, in order to be able to give an opinion on the question of environmental preference, geographical distribution, ability to compete and other problems.

The starting-point of this discussion should be a brief look at the environmental requirements of the individual species, with special reference to salinity tolerance, and to give particulars of the numerous individual works (inter alia SPOONER 1947, SEGERSTRÅLE 1947, 1959, KINNE 1954, 1959, DEN HARTOG 1964, MOVAGHAR 1964, STOCK 1967, DENNERT et al. 1969, PINKSTER et al. 1970).

Among the five species under consideration, G. locusta is a more marine form, living in the vegetation-rich, flat, littoral region of the temperate east Atlantic area. To the south it occurs adjacent to G. oceanicus. In regions where they both overlap, as for example in the Baltic, almost the same areas are colonised. G. oceanicus is an arctic boreal form of the Atlantic region and prefers the coastal, shallow water, but tolerates a greater freshening than G. locusta. G. salinus lives in brackish waters of the most different types, in which considerable salt content fluctuations can occur, although the species occurs also in purely marine regions. G. zaddachi is, like G. salinus, a strongly euryhaline species. It penetrates further in fresh water than the latter and avoids waters of the euhaline region. In the upper part of river estuaries G. salinus is in general replaced by G. zaddachi, where this species even reaches freshwater zones.

Finally G. duebeni is a strongly euryhaline and eurythermal brackish-water form, which among the Gammaridae under consideration possesses the greatest ecological potential. It shows a wide distribution on the north Atlantic coasts, and dwells in a great many different environments. G. duebeni is a characteristic representative of the "rockpool" fauna, colonises brackish pools, canals, ponds, the margins of estuaries and even hyperhaline waters. In the open sea, however, this form occurs only exceptionally. In addition G. duebeni could also exist in freshwater biotopes, where its occurrence is not only limited to the coastal region, but in Ireland and Brittany is also encountered in fresh water distant from the coast with a relatively low NaCl content. SUTCLIFFE & SHAW (1968) showed by means of the varying ability for sodium regulation, that the Irish freshwater population represents an individual physiological race compared with the brackish-water species, although SUTCLIFFE (1971) reports doubt, as to whether there really are genotypically linked characteristics, which justify speaking about two physiological races. STOCK & PINKSTER (1970), however, also found morphological peculiarities and distinguished the brackish-water form G. duebeni duebeni from the freshwater form G. duebeni celticus, as a subspecies.

From the above-mentioned data on the habitat preference and distribution it emerges that the individual species have colonised different ecological niches, which are quite well-defined by their tolerance in relation to the factor of salt content. Depending on the special factors of a biotope, their environments can indeed also overlap. While G. locusta mostly occurs alone - apart from occasional association with G. oceanicus and G. salinus - the distribution of G. oceanicus, G. salinus and G. zaddachi can regionally overlap, as in the Baltic area. In secluded, brackish water the coexistence of G. salinus, G. zaddachi and G. duebeni is also possible; in estuaries at any rate G. duebeni avoids competition with these species and colonises parts of the eu- and supralittoral, while G. salinus and G. zaddachi only occur in the lower eulittoral and sublittoral (DEN HARTOG 1964). Both the last-named species frequently occur sympatrially, while G. duebeni, apparently on the ground of its poorer competitive ability, but also as a consequence of its greater tolerance with regard to abiotic environmental factors, is forced into biotopes, in which the other *Gammarus* species are not able to exist (cf. KINNE 1959). As however the interspecific competitive ability cannot alone be explained by the physiological efficiency of a species, the different aspects of this question complex should not be discussed further.

If one places the different metabolic rates of the individual euryhaline Gammaridae in relation to the environments which they prefer, it becomes understandable that *G. locusta*, as a species with the relatively highest oxygen requirement, is principally distributed in oxygen-rich, open water, while *G. duebeni*, as the form with the lowest respiratory intensity, finds an opportunity to live in biotopes with extreme environmental conditions. The other three species however assume an intermediate position, in which they are not so clearly demarcated amongst each other, and where at any rate *G. oceanicus* is contrasted more clearly with *G. salinus* and *G. zaddachi* than both the last-named species are with each other.

A special adaptive significance is due to the ability of *G. duebeni* to adjust to a sudden change in salt content without lasting alterations to its metabolic intensity. This property should be seen in the light of the occurrence of this species in regions with especially strong variations in salinity. In connection with the physiological ability for adaptation to salt content, *G. locusta* on the other hand is shown to be at the other extreme. From the considerable rise of respiratory intensity after a change from a concentrated to a dilute medium it will be seen that the metabolic economy of this species is chronically burdened by a salinity stress of this kind. This circumstance offers an explanation why *G. locusta* does not penetrate poikilohaline estuaries, but prefers homoiohaline regions. Hence this species lives predominantly in the euhaline region; it could only progress to salt-ppor zones in a relatively stable medium, such as the Baltic, where there are very few non-periodical fluctuations in salinity.

The other three species show a similar physiological behaviour in relation to a change in salt content and in this respect also assume a central position. On the whole the compensatory ability of *G. zaddachi* appears to be somewhat better than that of G. salinus, if the metabolico-physiological differences between both these species are shown to be very small. This result corresponds with other data on the similarity of functional characteristics of both species, as investigations on the intansity of growth, moulting sequence, heart frequency and duration of the development of the embryo (KINNE 1961) as well as radiation resistance (HOPPENHEIT 1969) have shown. Although definite differences in ecological requirements can be recorded between G. salinus and G. zaddachi, their great structural similarity, especially of the young animals (cf. DENNERT et al. 1969), agrees with the opinion voiced by KINNE (1961), that they embody two comparatively young species, which evidently have only attained their genetic independence relatively late.

SUMMARY

1. The respiratory metabolism of the euryhaline amphipods Gammarus locusta (L.), G. oceanicus SEGERSTRÅLE, G. salinus SPOONER, G. zaddachi SEXTON and G. duebeni LILLJEBORG was studied polarographically in a flow-through apparatus at a temperature of 15°C.
2. For all five species the relationships between oxygen consumption (y) and body weight (x) were investigated in dependence on salt content (30°/oo and 10°/oo), and the parameters of the allometric functions $y = a.x^{b}$ were determined (cf. Tab. 1).
3. The regression coefficient b, which characterises the dependence of the metabolism on size, shows differences which are characteristic of the species, and is dependent on salt content. This is marked – with the exception of G. duebeni – through higher values at a salinity of 30°/oo, than at 10°/oo, and is significantly different in all cases. The dependence of the metabolism on size lies in the region between weight and surface proportionality respectively, and even below this ($b = 1-0.6$).
4. An interspecific comparison of the factor a, which characterises the respiratory size calculated out of the total measured values, from the unit of weight, gives the result that the metabolic intensity in the succession from G. locusta through G. oceanicus, G. salinus, G. zaddachi to G. duebeni progressively decreases. The average oxygen requirement of shrimps with

a living weight of 0.1 g. reaches from 17.1 cu.mm.^{3}/h (G. locusta) to 7.1 cu.mm.^{3}/h (G. duebeni) in 30°/oo.

5. The variations of the oxygen consumption after an abrupt change in salinity and a period of adaptation to the salt content were investigated from comparative viewpoints. Depending on the salinity tolerance of the individual species there followed a transition from 10°/oo to 30°/oo, from 30°/oo to 45°/oo, from 30°/oo to 10°/oo and from 10°/oo to 3°/oo.
6. With a change from a dilute to a concentrated medium, the process of adaptation takes place substantially more rapidly and is linked with smaller variations in respiratory intensity, than for transition in the reverse direction. The times of adaptation up to the attainment of a new, constant metabolic level lie between 3 and around 30 hours. G. duebeni possesses a better compensatory ability than the other species, amongst which G. locusta shows the relatively smallest regulatory capacity.
7. The rate of metabolism falls on deprivation of food. Measurements on G. oceanicus have shown that after 20–24 hours the respiratory intensity diminished by c. 15% compared with the initial values, and also grows only a little less within the following 5 days.
8. The metabolic activity in adult shrimps amounts to about 2½–3 times the value for rest. Under basic metabolic rate conditions there can appear a pronounced rhythm of the ventilatory movements of the pleopoda.
9. During moulting the oxygen intake increases from 2.2 to 3.9 times the average normal consumption. For G. locusta were registered the highest increases in metabolism, and for G. duebeni the lowest.
10. Different metabolico-physiological aspects, especially the problem of the salt content adaptation and the osmo- and ion-regulatory processes linked with it, are discussed. The metabolic efficiency of the individual Gammarus species are compared and placed in relation to their environmental requirements.

Prozesse, werden diskutiert. Die Stoffwechselleistungen der einzelnen *Gammarus*-Arten werden verglichen und zu ihren Umweltansprüchen in Beziehung gesetzt.

Danksagungen. Herr Prof. Dr. F. Krüger gestattete mir, die rechnerischen Auswertungen an einem Wang Mini-Computer vorzunehmen, der ihm als Leihgabe von der Deutschen Forschungsgemeinschaft zur Verfügung gestellt wurde. Die Kollegen Dr. M. Hoppenheit und Dr. D. Siebers förderten die Fertigstellung der Arbeit durch kritische Diskussionen. Fräulein M. Mühlenkamp assistierte bei der Durchführung der Experimente und deren Auswertung. Herr J. Marschall übernahm die Anfertigung der Zeichnungen. Allen genannten Mitarbeitern der Biologischen Anstalt Helgoland bin ich für ihre Hilfe sehr zu Dank verpflichtet.

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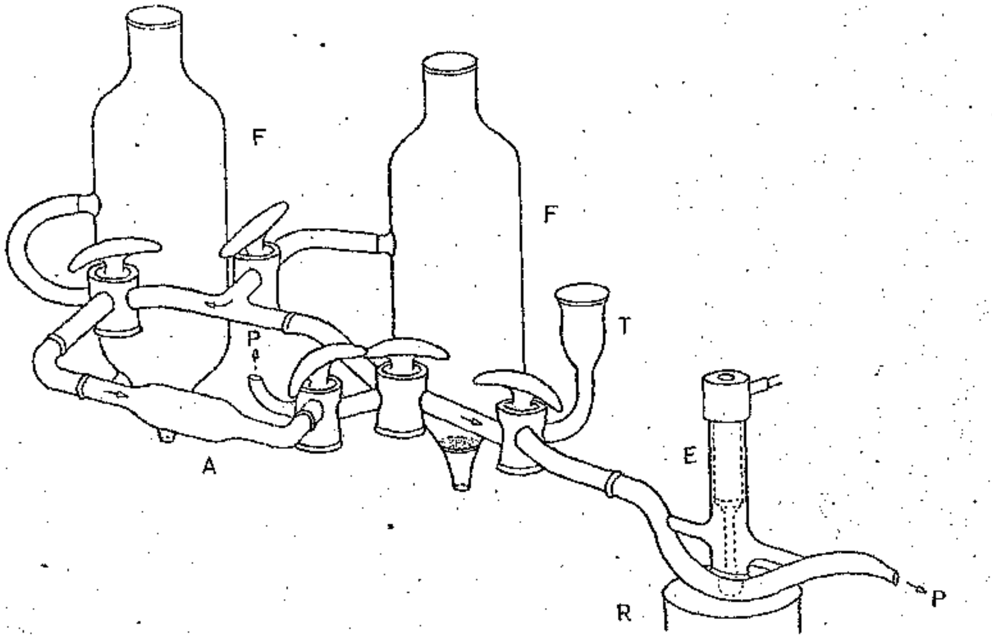
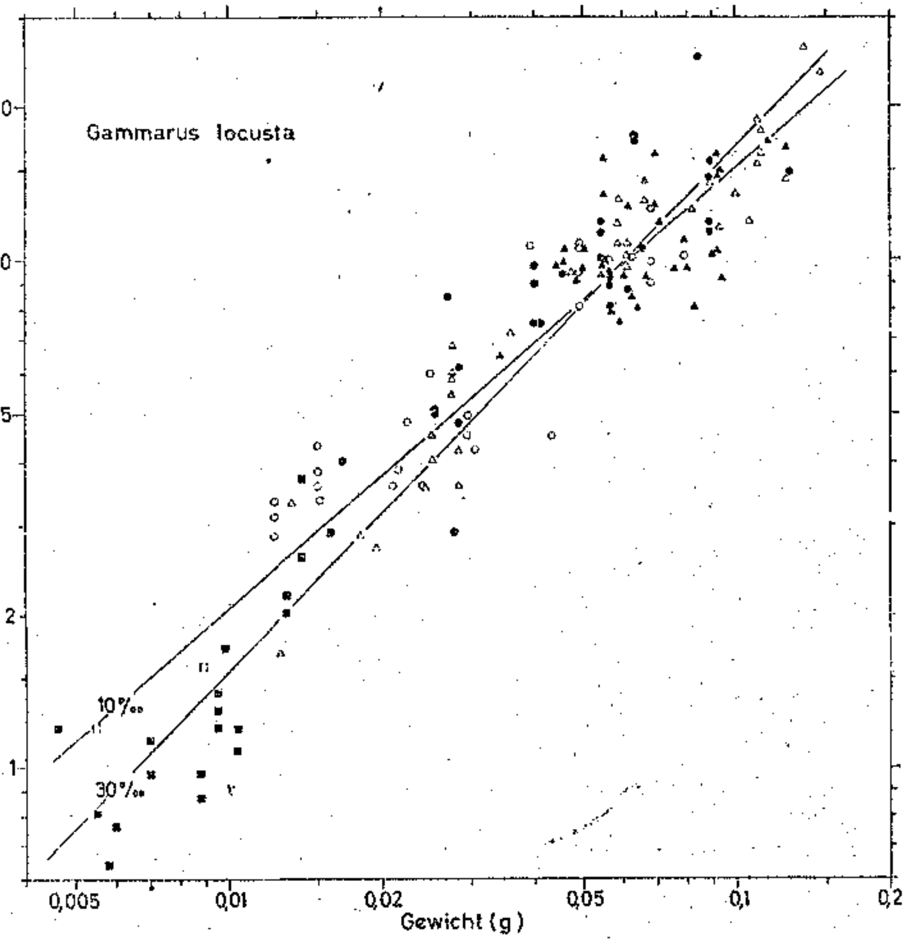
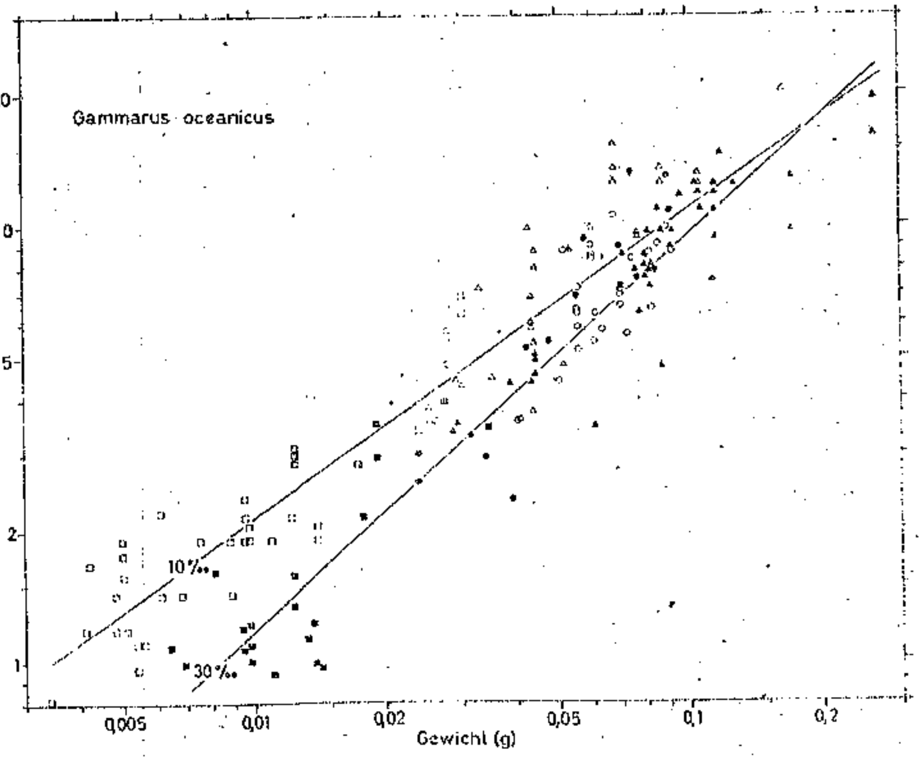


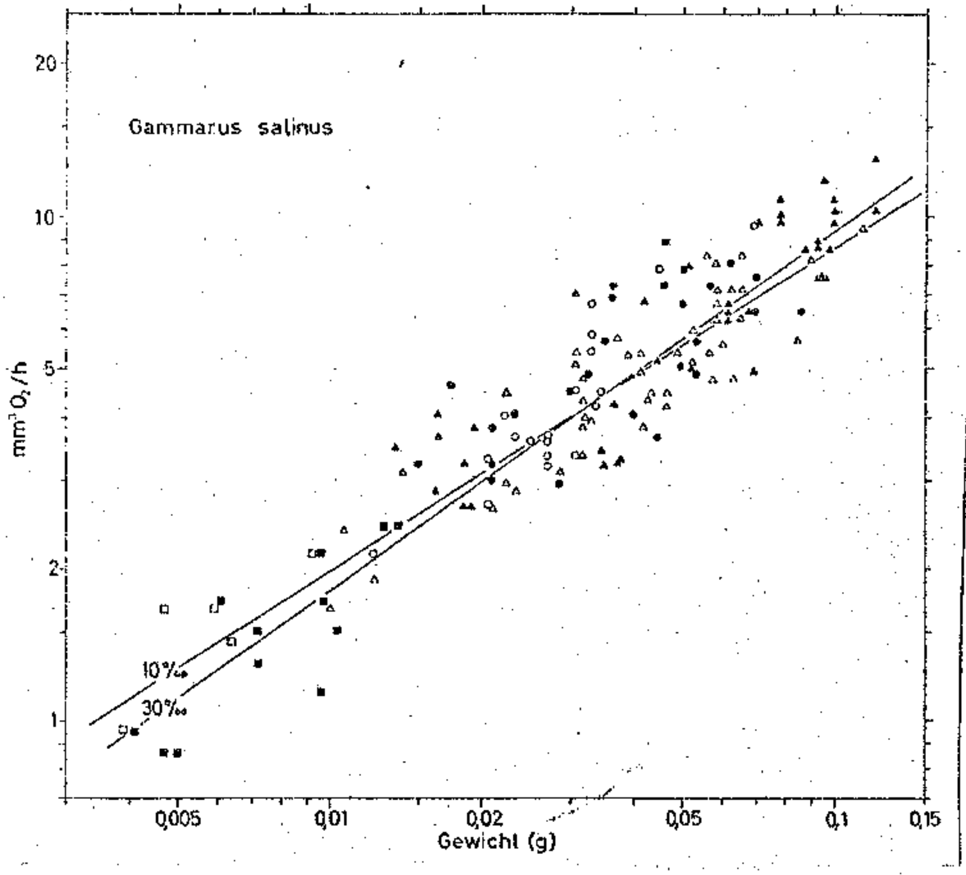
1. Plan of the flow-through apparatus with the respiration chamber (A), p02 electrode (E), magnetic stirrer (R), two cohering bottles (F) to equalise the experimental medium with oxygen in the air and filler funnel (T) for the intake of oxygen-free water for the calibration. The arrows give the direction of flow for the experimental medium delivered through the pumps. By means of polished ball sockets (Kugelschliffe) the individual elements of the glass flow-through system are connected to each other. The metal clamps used for fixing, and other support devices have not been shown.



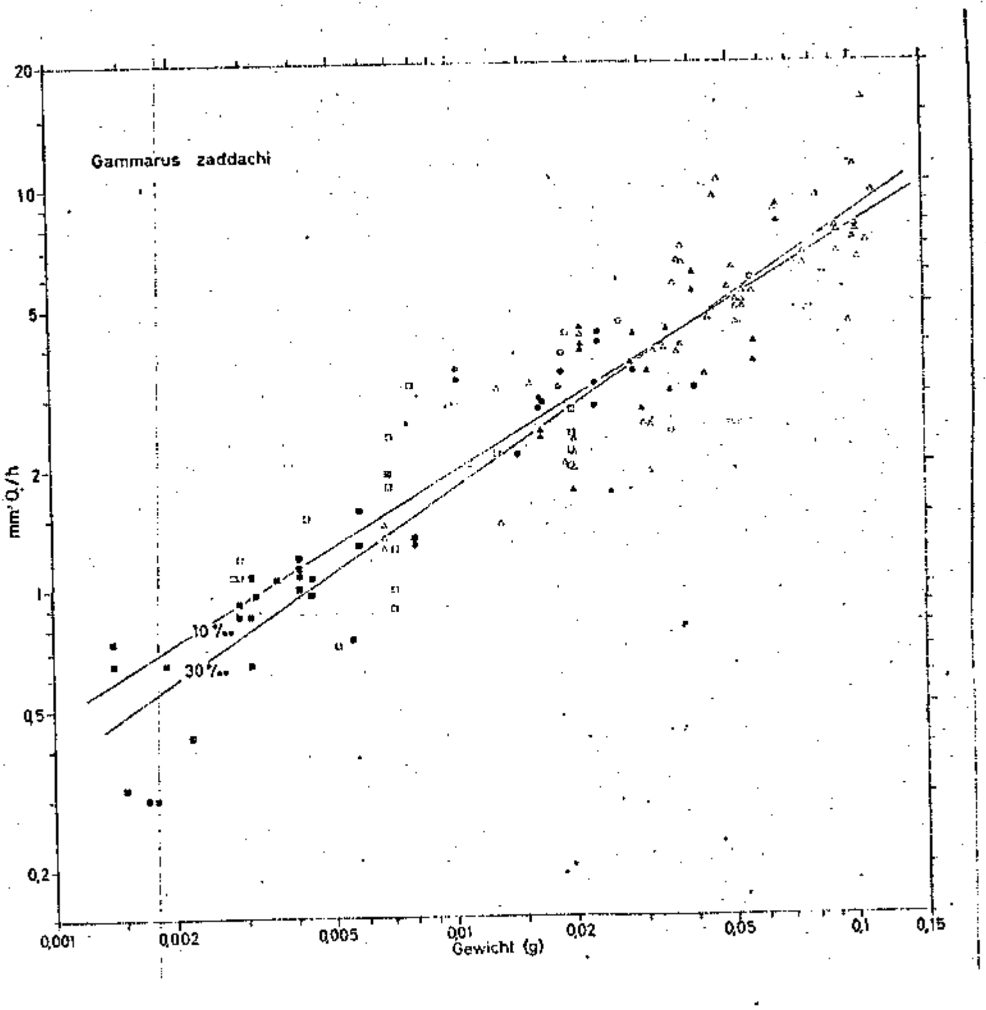
2. Gammarus locusta. Dependence of the oxygen consumption on body weight (fresh weight) at 30^{o}/oo and 10^{o}/oo (15^{o}C) in double-logarithmic representation. The regression lines calculated from the measurement data have been shown for both salinities.
Symbols of the 30^{o}/oo stage: black square - young animals not yet sexually differentiated; black circle - females; black triangle - males.
(These symbols are in white for the 10^{o}/oo stage).



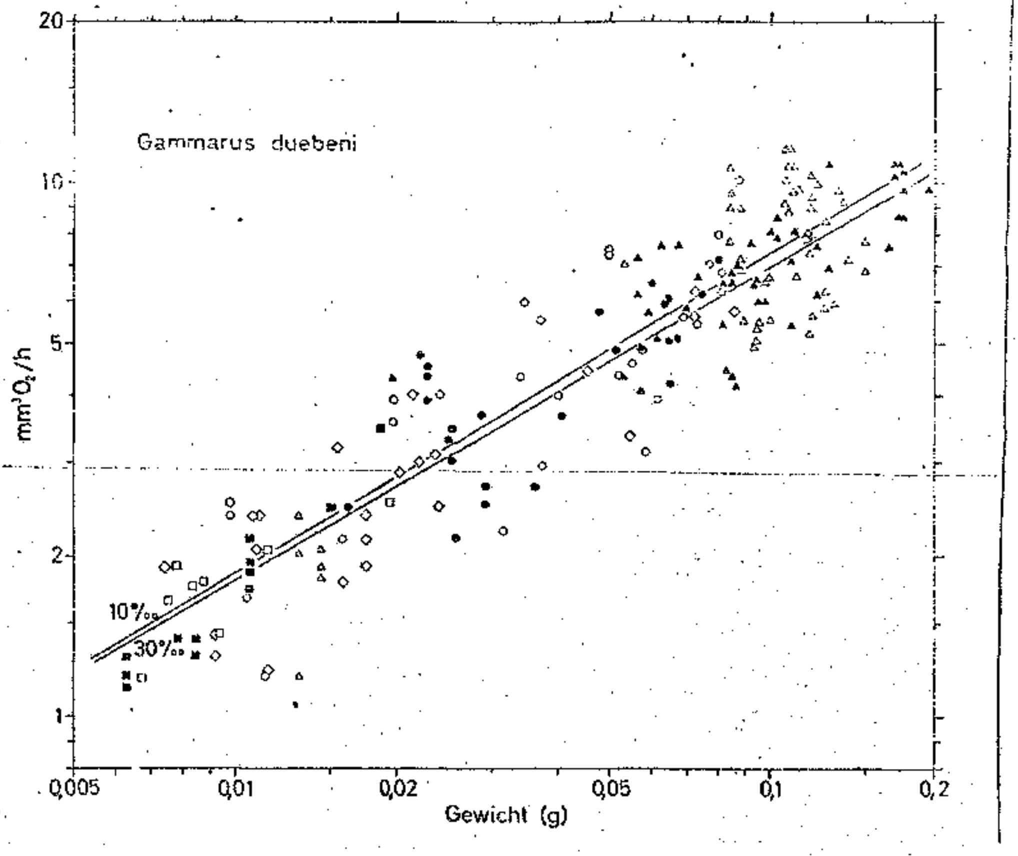
3. Gammarus oceanicus.. Dependence of the oxygen consumption on body weight at 30^{o}/oo and 10^{o}/oo and at 15^{o}C. (Cf. explanations for fig. 2).



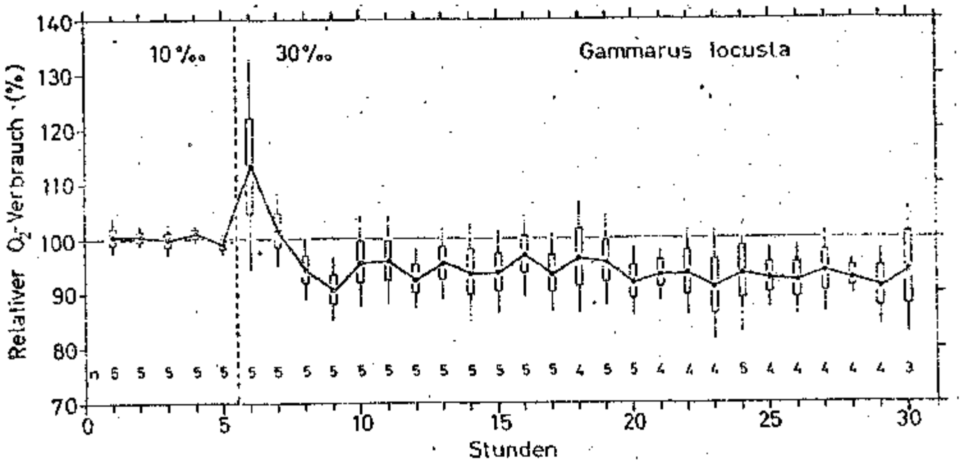
4. <u>Gammarus salinus</u>. Dependence of the oxygen consumption on body weight at 30°/oo and 10°/oo and at 15°C.



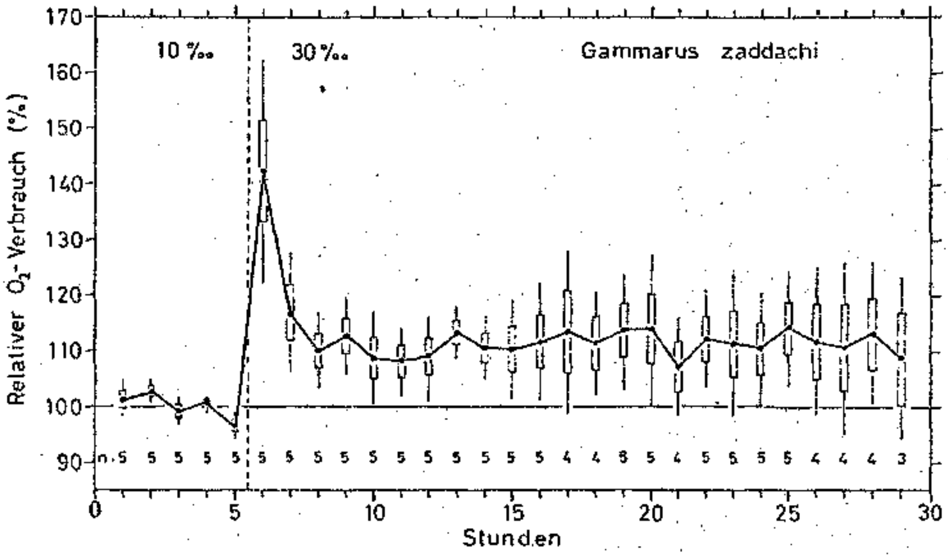
5. <u>Gammarus zaddachi</u>. Dependence of the oxygen consumption on body weight at 30°/oo and 10°/oo at 15°C.



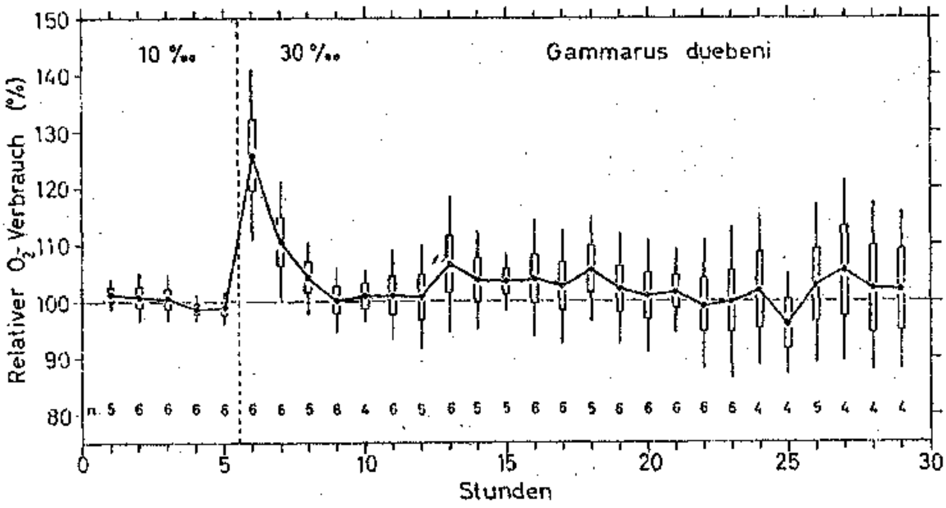
6. Gammarus duebeni. Dependence of the oxygen consumption on body weight at 30°/oo and 10°/oo at 15°C. (White diamond) - measurements for the young animals and females infected with Thelohania hereditaria.



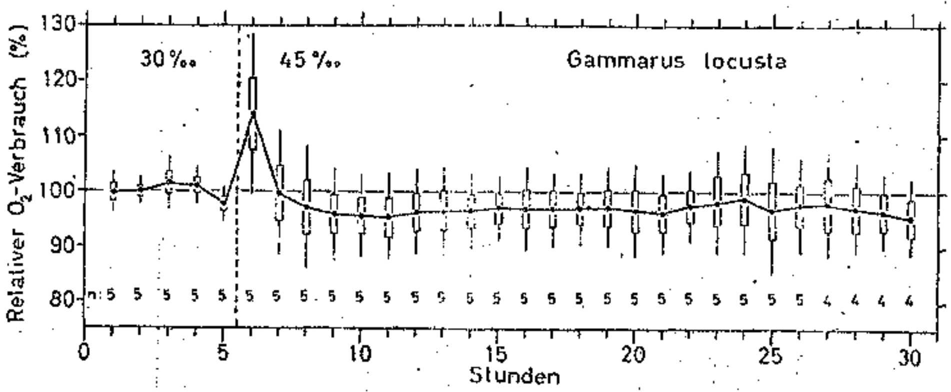
7. Gammarus locusta. (Males, 0.04-0.06 g.) Relative oxygen consumption (at 15°C) after an abrupt change in salinity from 10°/oo to 30°/oo as a function of time. The average error of the average value (vertical rectangles) and the standard deviation (vertical lines) were calculated for the metabolic data reproduced as relative values. n = number of experimental animals.



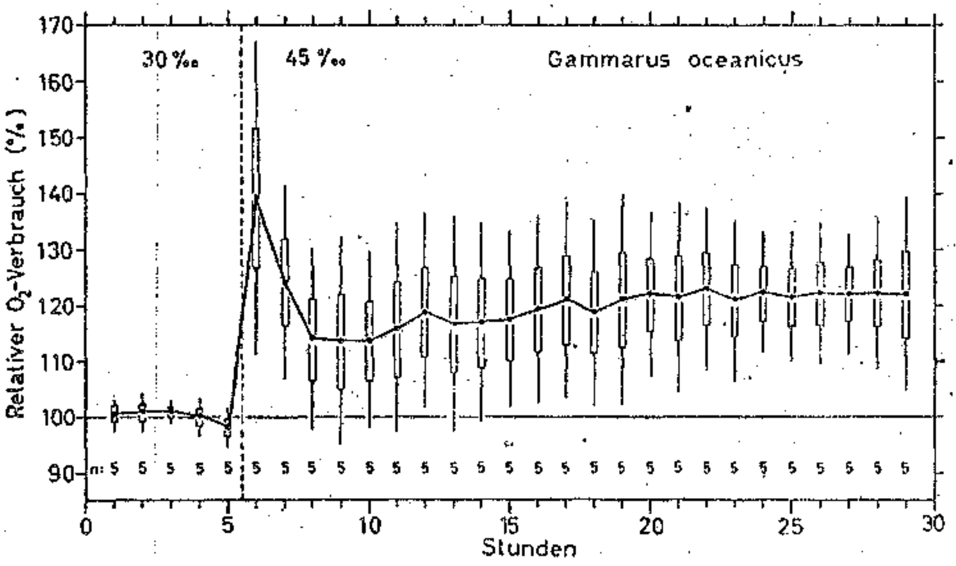
8. Gammarus zaddachi. (Males, 0.02-0.05 g.) Relative oxygen consumption (at 15°C) after an abrupt change in salinity from 10°/oo to 30°/oo. (Cf. explanations to Fig. 7).



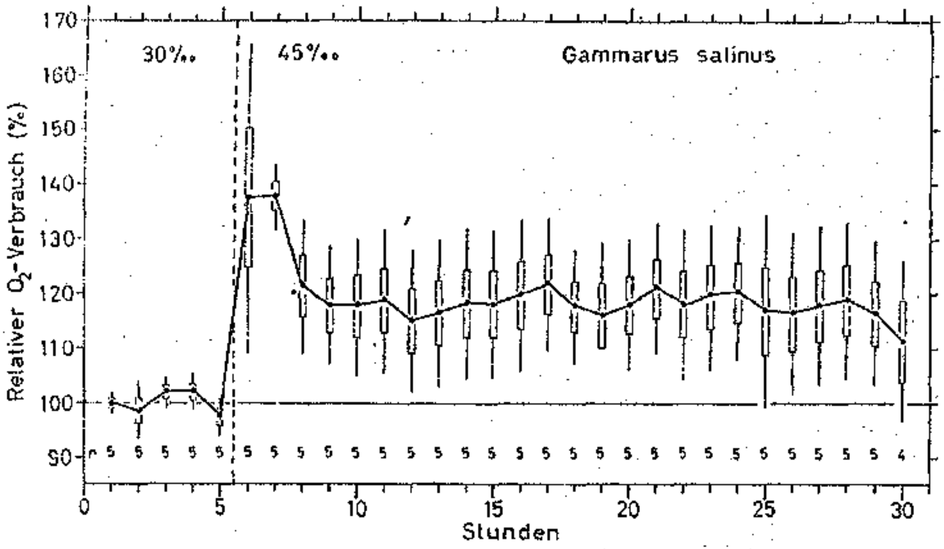
9. Gammarus duebeni (males and females, 0.06-0.09 g.). Relative oxygen consumption (at 15°C) after an abrupt change in salinity from 10°/oo to 30°/oo.



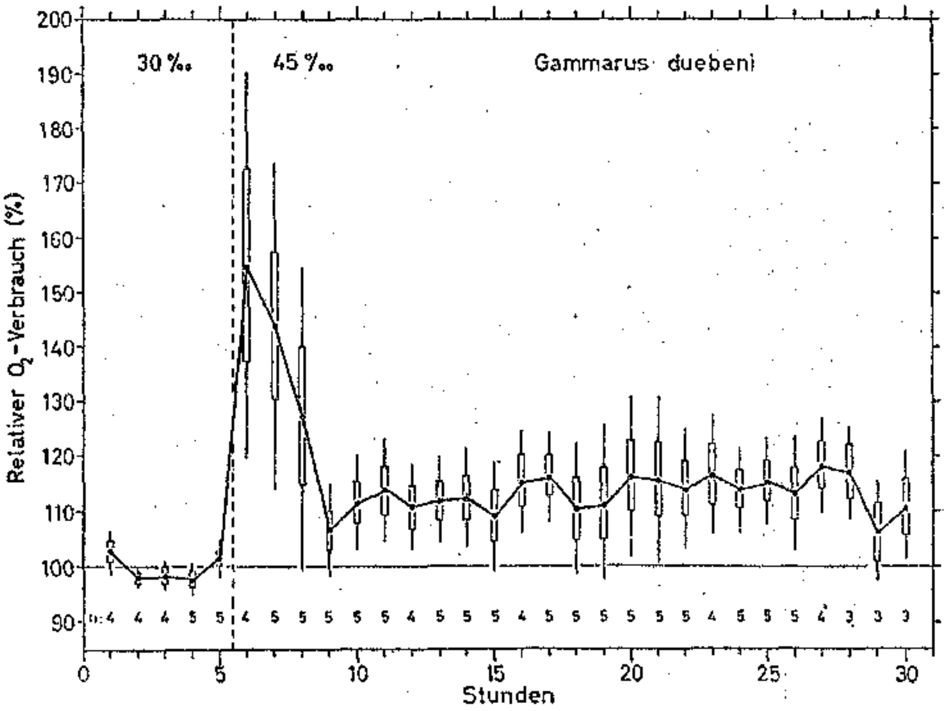
10. Gammarus locusta (males and females, 0.06-0.07 g.). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 30°/oo to 45°/oo.



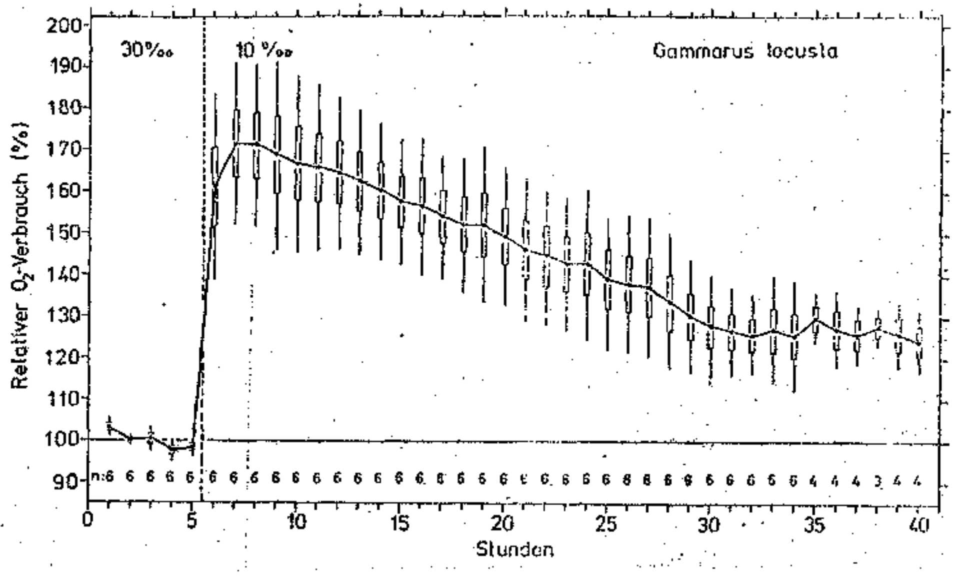
11. Gammarus oceanicus (males and females, 0.03-0.04 g.). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 30°/oo to 45°/oo.



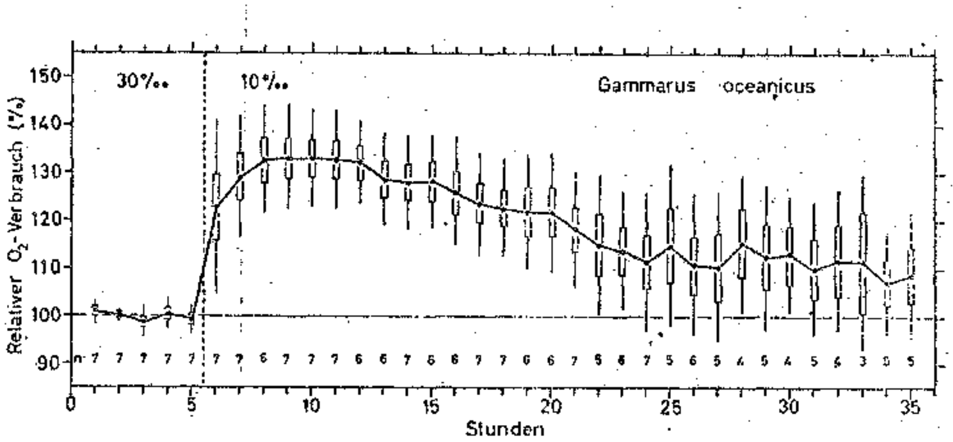
12. Gammarus salinus (males, 0.03-0.04 g.). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 30°/oo to 45°/oo.



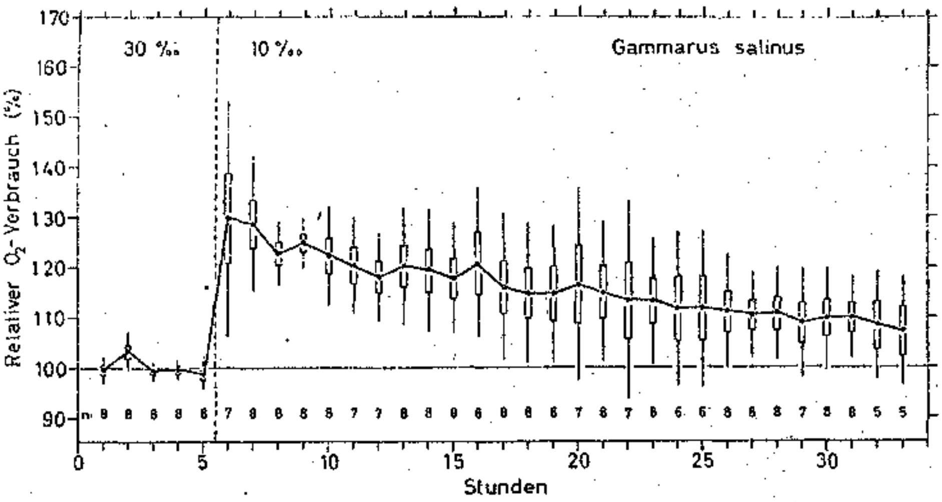
13. Gammarus duebeni (males and females, 0.04-0.08 g.). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 30°/oo to 45°/oo.



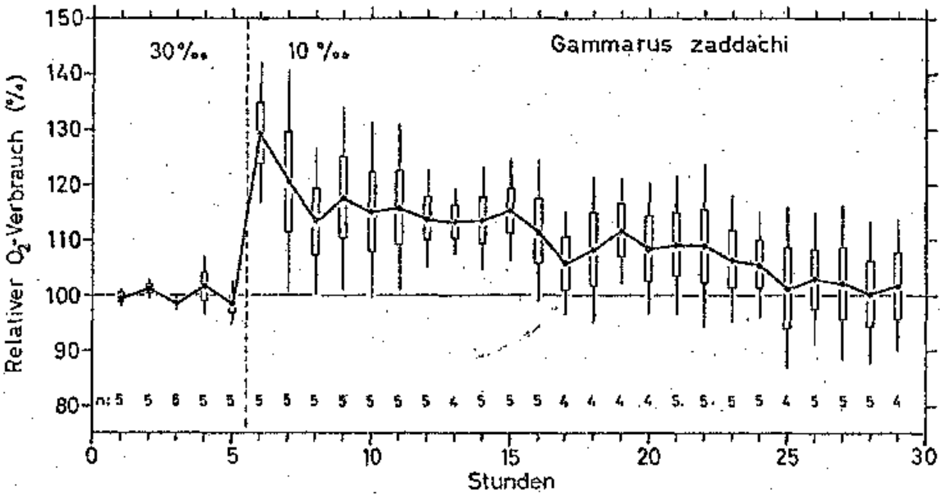
14. Gammarus locusta (males, 0.04-0.06 g.). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 30°/oo to 10°/oo.



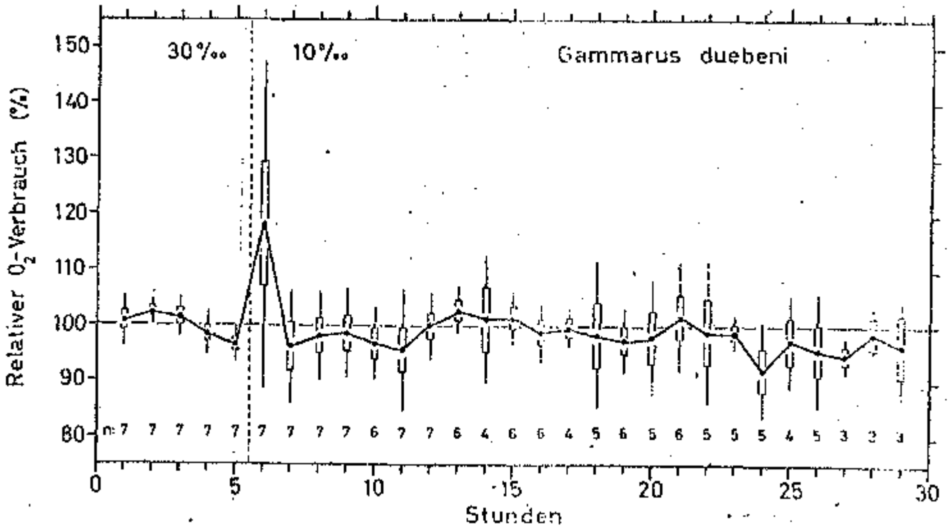
15. Gammarus oceanicus (males and females, 0.07-0.08 g.). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 30°/oo to 10°/oo.



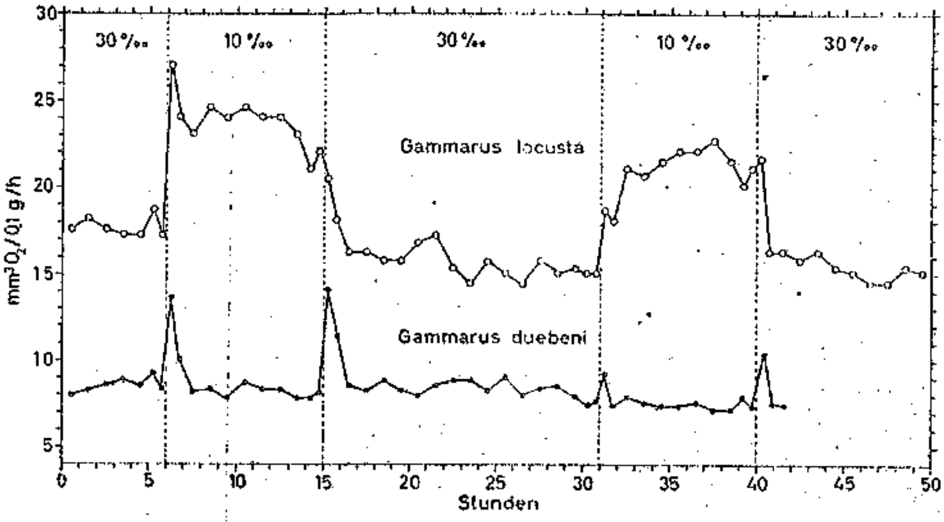
16. Gammarus salinus (males and females, 0.03–0.05 g.). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 30°/oo to 10°/oo.



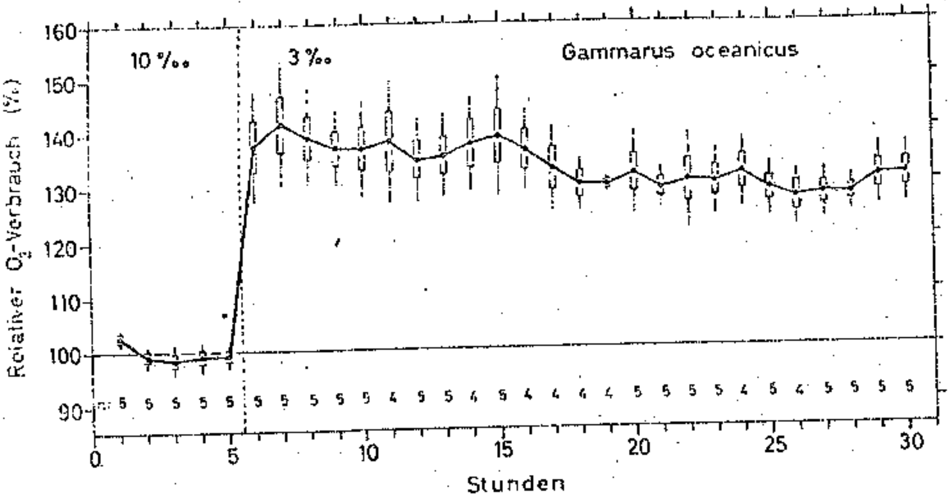
17. Gammarus zaddachi (males, 0.03–0.04 g). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 30°/oo to 10°/oo.



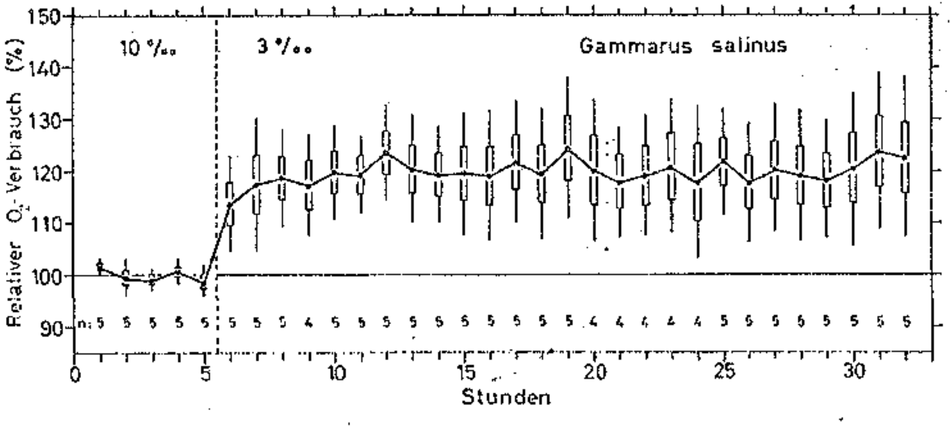
18. Gammarus duebeni (males and females, 0.07–0.1 g). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 30°/oo to 10°/ooo



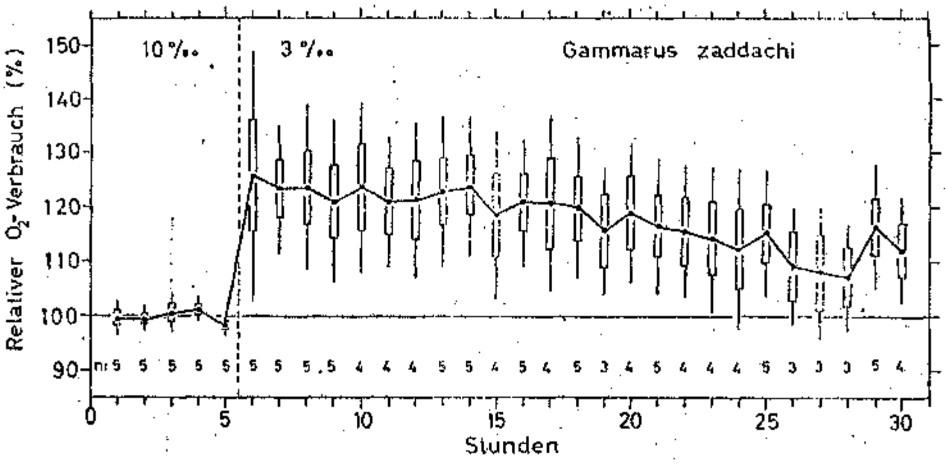
19. Oxygen consumption (at 15°C) of a female of Gammarus locusta (0.089 g.) and a male of Gammarus duebeni (0.091 g.) after several abrupt salinity changes from 30°/oo to 10°/oo and reverse.



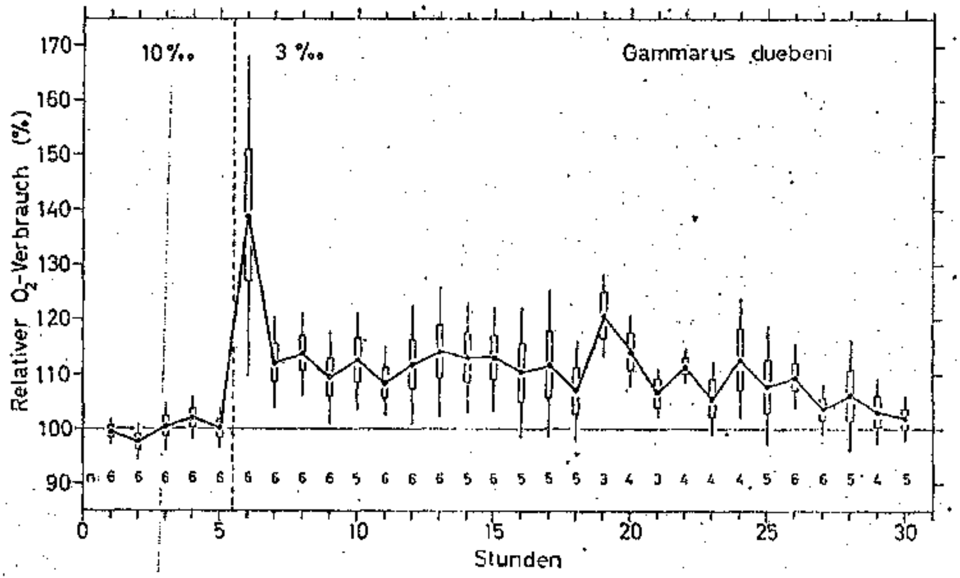
20. Gammarus oceanicus (females, 0.06–0.08 g.). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 10°/oo to 3°/oo.



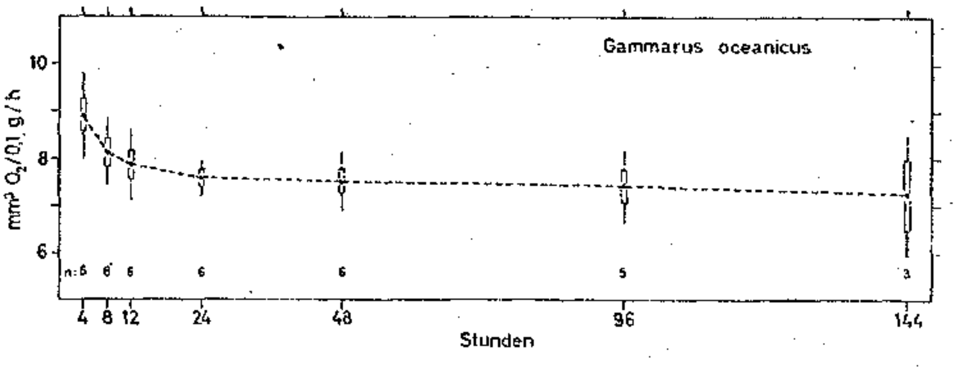
21. Gammarus salinus (males and females, 0.06–0.08 g.). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 10°/oo to 3°/oo.



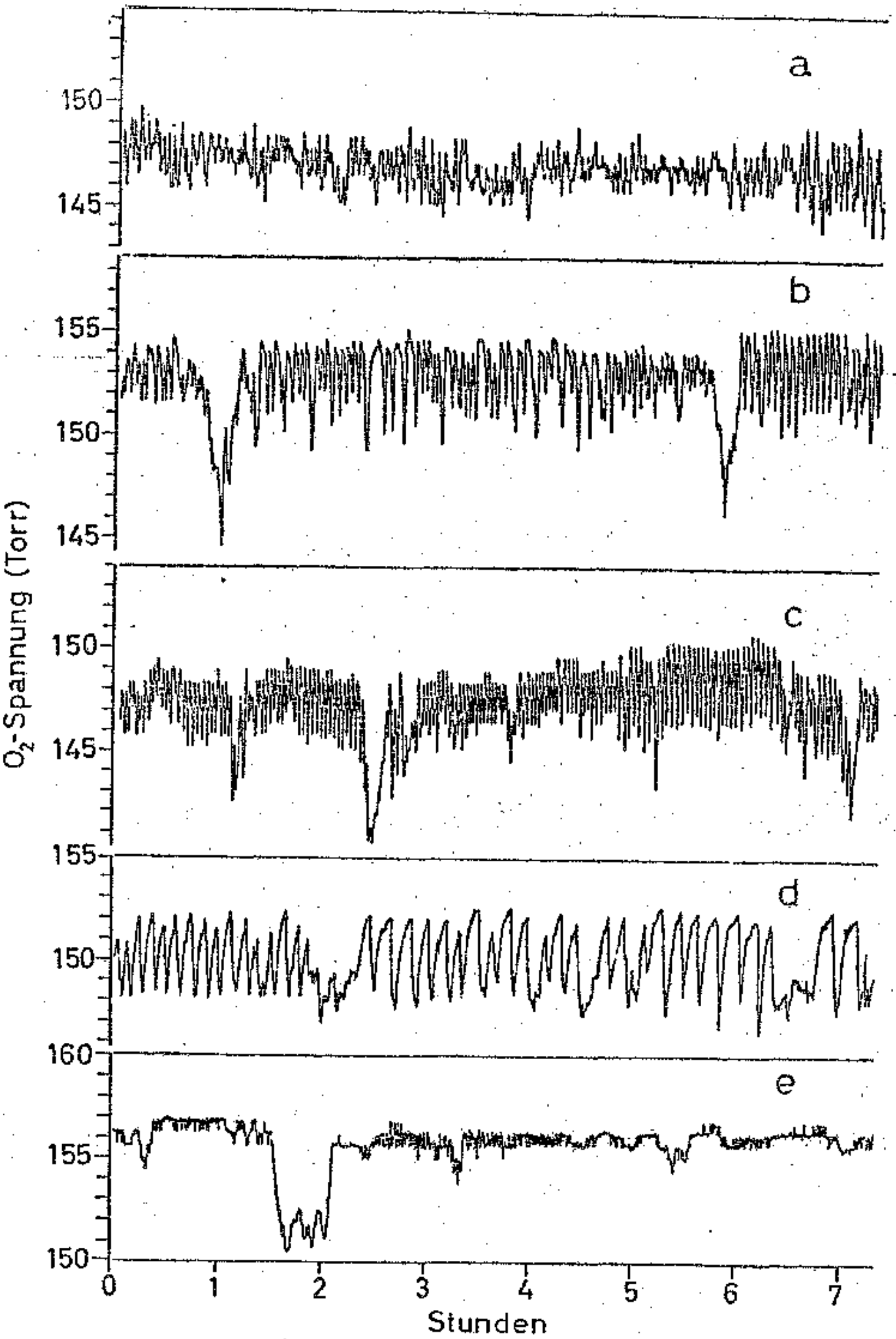
22. Gammarus zaddachi (males, 0.10-0.11 g.). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 10°/oo to 3°/oo.



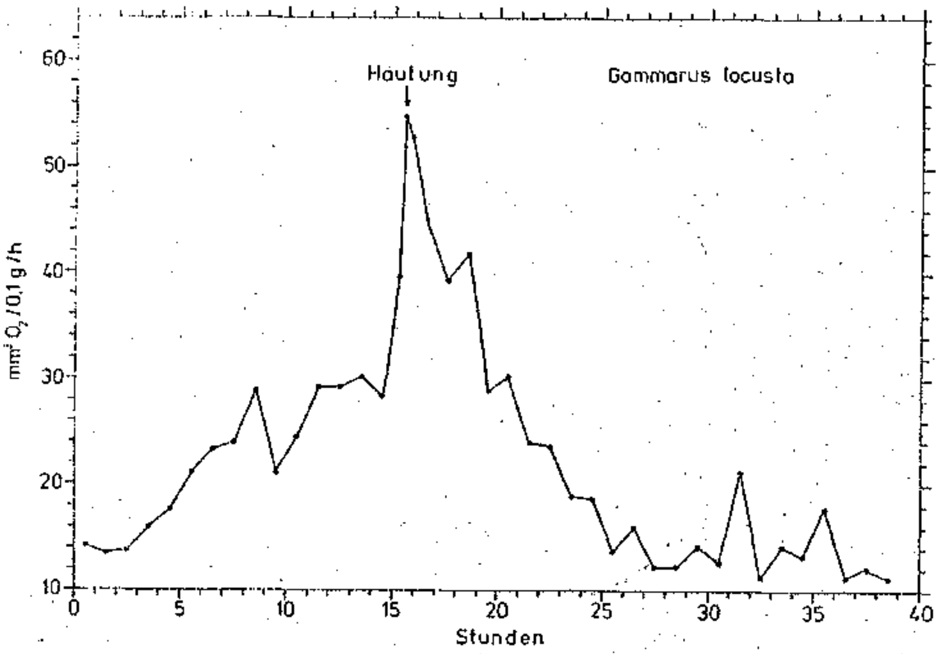
23. Gammarus duebeni (males, 0.09-0.11 g.). Relative oxygen consumption (at 15°C) after an abrupt salinity change from 10°/oo to 3°/oo.



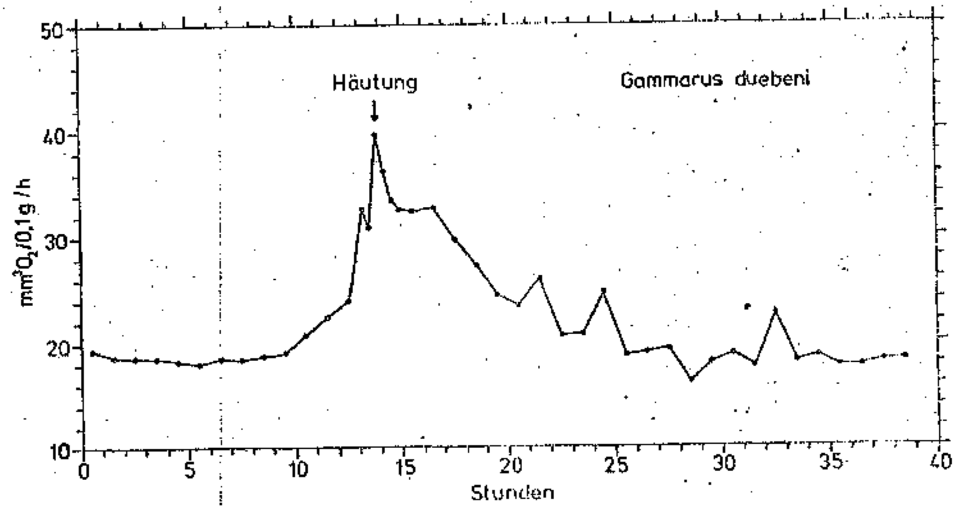
24. Gammarus oceanicus (males, 0.07-0.09 g.). Variations in oxygen consumption (30°/oo, 15°C) during the course of a six-day period of starvation.



25. Recorded examples of different experimental animals with a clearly pronounced ventilation rhythm in parts. a – Gammarus locusta (females 30°/oo); b – G. oceanicus (male, 30°/oo); c – G. salinus (female, 30°/oo); d – G. duebeni (female, 10°/oo); e – G. duebeni (young animal, 30°/oo). The regularity of the curve is in some cases (b,c,e) broken by short-term phases of activity, which cause a rise in oxygen intake. The lines above the respiratory curves reproduce the oxygen pressure of air-saturated water, in dependence on the actual air pressure.



26. Gammarus locusta (female, 30°/oo). Oxygen consumption (at 15°C) during the process of moulting.



27. Gammarus duebeni (female, 10°/oo). Oxygen consumption (at 15°C) during the moulting process.

Tables

1. Parameters of the allometric relation $y = a.x^b$ between body weight (fresh weight) and oxygen consumption (cu.mm.^{3} O$_2$/g/h) for five euryhaline Gammarus species depending on salt gontent (15 °C). a = segment of the ordinates; s_a = standard deviation of the ordinate segment a; b = regression coefficient; s_b = standard deviation of the regression coefficient b; $s_{y.x}$ = standard error of the regression line; r = correlation coefficient; n = number of the pair values.

Species	Salinität (‰)	a	s_a	b	s_b	$s_{y \cdot x}$	r	n
G. locusta	30	180,33	13,37	1,024	0,035	1,327	0,956	84
	10	113,71	13,27	0,868	0,035	1,234	0,954	64
G. oceanicus	30	76,58	12,85	0,896	0,032	1,295	0,956	75
	10	54,25	12,50	0,702	0,024	1,281	0,947	97
G. salinus	30	48,96	13,03	0,699	0,032	1,269	0,930	79
	10	37,57	13,27	0,632	0,033	1,206	0,920	69
G. zaddachi	30	40,55	15,10	0,672	0,035	1,357	0,931	59
	10	31,77	13,87	0,609	0,036	1,380	0,887	80
G. duebeni	30	26,92	12,08	0,582	0,025	1,231	0,934	80
	10	29,99	12,59	0,608	0,031	1,308	0,907	86
G. duebeni (juv. + ♀♀)	10	30,63	16,63	0,607	0,054	1,316	0,888	36
G. duebeni (*Thelohania*-infizierte juv. + ♀♀)	10	24,35	18,62	0,570	0,062	1,257	0,896	23

2. Data on the variation in respiratory intensity during moulting for different Gammarus species (15 °C).
(Headings): species, sex, fresh weight (mg), salinity (°/oo), rise of the QO$_2$ before moulting (hrs.), fall of the QO$_2$ after moulting (hrs.), QO$_2$ during moulting (%) related to normal values (= 100%).

Species	Geschlecht	Frischgewicht (mg)	Salinität (‰)	Anstieg des Q_{O_2} vor der Häutung (Std)	Abfall des Q_{O_2} nach der Häutung (Std)	Q_{O_2} während der Häutung (%) bezogen auf Normalwerte (= 100 %)
G. locusta	♀	71,0	30	12	11–13	310
	♀	57,1	30	12	~ 12	390
	♀	12,7	30	5–7	8	230
G. oceanicus	♂	14,5	30	~ 5	10–12	260
G. salinus	♂	46,5	30	?	~ 8	270
	♀	27,4	30	6–7	~ 10	240
G. zaddachi	♂	113,2	10	?	30	380
G. duebeni	♂	67,6	30	4–5	15	220
	♀	49,4	10	6	14	220
	♀	34,8	10	10	~ 20	310

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