OXYGEN ECONOMY OF *COXELMIS NOVEMNOTATA* (KING) (COLEOPTERA, DRYOPIDAE).

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(Five Text-figures.)

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Introduction.

Coxelmis novemnotata (King) is a member of the sub-family Helminae, family Dryopidae. It occurs commonly on submerged logs in freshwater streams around Sydney. It is never found in water which is actually stagnant; the oxygen content of waters where it occurs is normally rather high (all determinations over 50% saturated). It appears never to come to the surface, but to carry on continuous oxygen exchange with the water in which it lives. Indefinitely-continued utilization of dissolved oxygen, with no direct or indirect contact with the surface, is exceptional for adult insects; it has been shown to occur by Szabó-Patay (1924) in *Aphelochirus aestivalis* F. (Hemiptera, Aphelochiridae), and suggested by Esaki and China (1927) for *Idiocoris* and *Paskia* (Hemiptera, Halotrephidae), and possibly for *Pontomyia natans* Edwards (Diptera).

Specimens used in the present work were collected in freshwater creeks at Galston Gorge, near Hornsby, N.S.W. They agree with the specific concept of *Coxelmis novemnotata* (King) C. & Z. elaborated by Carter and Zeck (1929); the author has also repeatedly compared specimens with material identified by the late H. J. Carter.

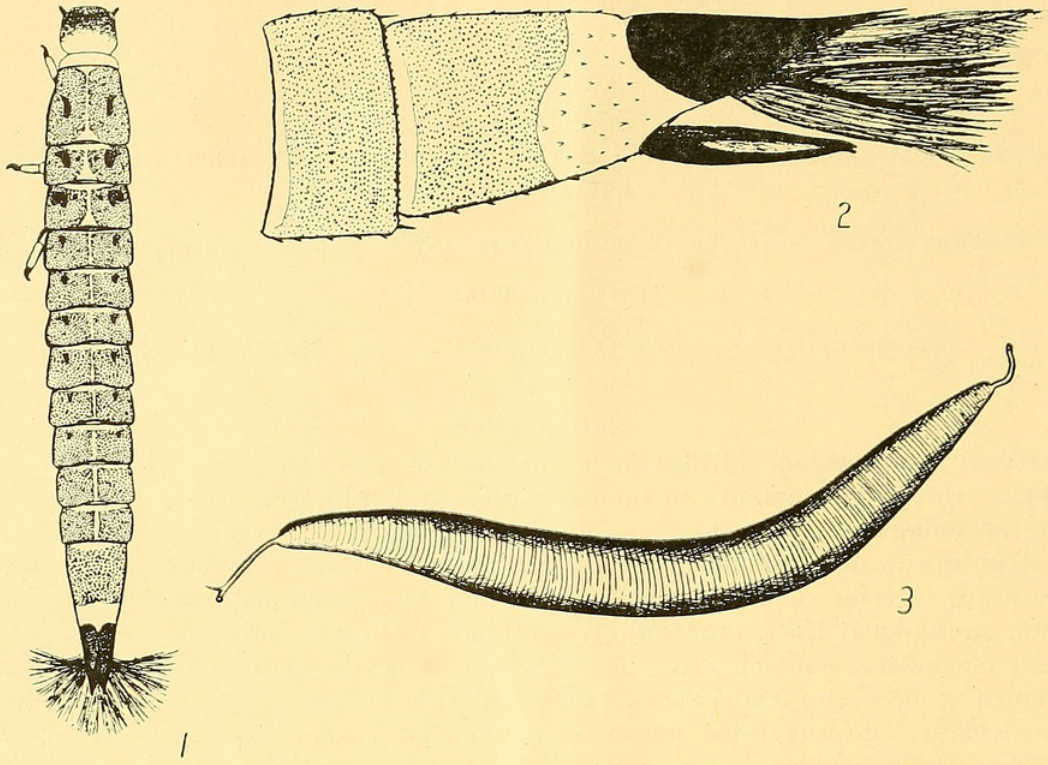
The larva. Figs. 1–4.

Larvae appeared in a culture of adults of *Coxelmis novemnotata* which had been kept for three months on dead wood submerged under running water. Larvae of exactly similar structure (Fig. 1), in all stages, have been collected in association with adults in the field (Galston Creek), where they form superficial channels on the surface of dead, submerged logs. They agree closely with the larvae of *Helmis pusillus* Lec., figured by Böving and Craighead (1931, Pl. 71, T). The conical ninth abdominal segment (Fig. 2) carries a ventral flap or operculum, which can be closed by muscle action and opened by pressure. When open, the tracheal gills, consisting of one mid-dorsal and two lateral tufts, are extruded. Each of the two main longitudinal tracheal trunks of the body bifurcates posteriorly, sending a component to the mid-dorsal group and a connection to the lateral group on that side. The gills are rhythmically extruded and retracted by the living larva.

Twelve large fusiform tracheae (Fig. 3) are grouped around, and parallel to, the alimentary canal in the anterior and middle region of the abdomen; in larvae of length 7–8 mm., these tracheae are 1·4 mm. long and 0·13 mm. in diameter. Each connects at one end, by means of a slender tracheole, to one of the two main tracheal trunks; at the other end, a fine tracheole arises, sending off capillaries which are applied to the alimentary canal. These twelve organs can be regarded only as gas reservoirs.

Each main tracheal trunk gives off a short, slender branch in the second thoracic segment (Fig. 4); this branch leads to a latero-dorsal spiracle, which may be non-functional. It could not be determined (by dissection) whether an actual opening was present, or merely a transparent area in the body-wall. At all events, the small size of this spiracle (0·01 mm. diameter), even if it is actually open, discounts any functional importance, and the larval tracheal system may be considered virtually if not actually closed.*

* The presence of at least one pair of spiracles is used as a criterion for the Helminae in the larval key of Böving and Craighead (op. cit., p. 45). This is not taken to imply that the spiracles are necessarily functional.



Figs. 1-3.—*Coxelmis novemnotata* (King), larva. 1, Dorsal view, × 12, anal gills fully extruded. 2, Lateral view of eighth and ninth abdominal segments, showing ventral operculum and three tufts of gills, × 30. 3, One of the twelve tracheal reservoirs from the abdomen, × 63.

The original formation of gas in the tracheae is probably caused by super-saturation due to a reduction in hydrostatic pressure, this being caused by exosmosis from the rigid closed tracheal system following muscular activity at eclosion, as described by Helson (1935) for *Stenoperla prasina* Newman (Perlaria, Eustheniidae). There is no reason for supposing that the oxygen economy of the larvae of *Coxelmis novemnotata* differs from that of any other purely aquatic insect larva with a closed tracheal system and external tracheal gills. Preliminary experiments suggest that the oxygen requirements of the largest larvae (length 7–8 mm.) are twice to three times those of the adults (*infra*).

THE ADULT. Fig. 5.

A. *Description, Observations and Qualitative Experiments.*

The average length of adults of *Coxelmis novemnotata* is 3 mm., the ventral surface occupying an average area of 0·028 cm.^{2} The ventral surface is covered with parallel adpressed hairs, spaced approximately 0·0025 mm. apart in the lateral regions, more sparse towards the mid-line. Except in the median areas, the ventral surface of submerged specimens is covered by a thin gas film; the absence of air from the mid-ventral region is approximately compensated by the air-films of the pubescent coxae, so that 0·028 cm.^{2} appears to be a fair estimate of the area of the gas-water interface. Dorsally, hairs are sparse and no film is present.

The ventral air-film is directly continuous with the sub-elytral air stores, which occupy approximately 0·06 cu. mm. Into the air stores open the spiracles, two thoracic and six abdominal. The last two pairs of spiracles (Fig. 5) are the largest, and they are connected to the longitudinal spiracular trunks by relatively thick tracheae (diameter 0·05 mm., compared with 0·02 mm. for the trunk). Six fine sub-parallel tracheoles are attached to the underside of each elytron, running longitudinally and abutting on the air stores. They seem unlikely to have any functional significance.

Adults freed from superficial moisture have an average weight of 1·4 mgm.; after 30 minutes in air, the average weight of the still-active beetles is 1·2 mgm. Sub-lethal drying reduces the average to 1·0 mgm. The second figure seems the truest estimate of the live weight. The average weight after treatment with strong caustic soda, followed

by washing and drying, is 0·2 mgm., giving the average weight of living tissue as 1·0 mgm. per beetle.

The adults occur on submerged logs, very often in such a position that access to the surface by means other than swimming or floating seems impossible. They are very inactive, adhering to the substrate by means of the over-developed tarsal claws. They are unable to swim, moving the legs to and fro aimlessly and ineffectively when detached from the substrate. By contraction of the body, some measure of controlled buoyancy seems to be effected, on the principle of the "Cartesian Diver". Field observations lead to the view that the adults normally never leave the water, or even come to the surface. When removed from water, they attempt to return, and, although they can survive for 30 minutes in air at 70% relative humidity at 17°C., 60 minutes' exposure causes death, no specimens recovering when returned to water after that time.

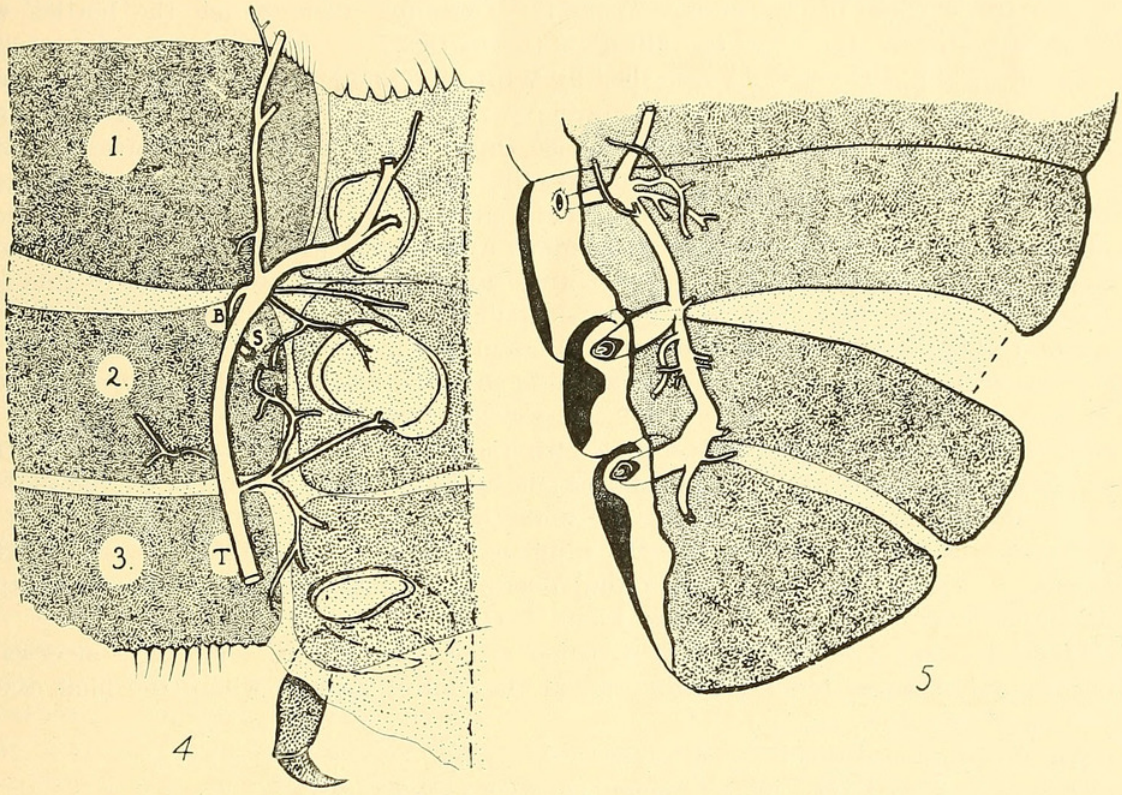


Fig. 4.—*Coxelmis novemnotata* (King), larva; left half of thorax viewed from above, the tergal region folded back to the left, × 48. (1, 2, 3, thoracic terga, from inside; *T*, left longitudinal tracheal trunk; *B*, small tracheal branch leading from trunk to *S*, reduced (? non-functional) spiracle.)

Fig. 5.—*Coxelmis novemnotata* (King), adult. Dorsal view of tip of abdomen, × 59; dorsal body-wall removed except for a small flap on the left; all organs except parts of the left half of the tracheal system removed. The two large posterior spiracles, and their thick connections to the longitudinal spiracular trunk, are visible. (All figures based on camera lucida outlines.)

Adults have been kept under running tap-water in the laboratory for nine months without any apparent injury, larvae appearing in the culture during that time; all access to air was prevented by a muslin cover. Carter and Zeck (op. cit., p. 50) record the keeping of adults submerged in an aquarium, aerated with aquatic plants, for five months.

It has been noticed that, in crowded cultures subject to oxygen lack, slightly increased temperatures often cause death; under the same conditions, muscular activity associated with lack of a suitable substrate produces the same results. When submerged in running tap-water, adults arrange themselves at random over the substrate; when placed in air-free water, they gradually arrange themselves at the surface or within 5 mm. of it.

It seems that all the indirect evidence is in favour of the view that adults of this species normally remain submerged continuously, and obtain their oxygen requirements from solution by means of the ventral air-film, which thus acts as a gill.

B. *Quantitative Experiments.*

Healthy adults of *Coxelmis novemnotata* were freed from superficial dirt by gentle rubbing between cotton wool, immersed for one minute in a 1% aqueous solution of mercuric chloride, and transferred to distilled water for immediate use. Several litres of distilled water, aerated by bubbling, were transferred to a large open container and kept well stirred. Weighing bottles (volumes 15-22 c.c.) were filled with this water, and into some bottles a known number of beetles was introduced, alternate bottles being kept as controls. All bottles were quickly sealed with complete exclusion of air bubbles, and sunk in a water-bath for temperature stabilization. The contents were mixed by inversion at the end of 3, 6, 18 and 24 hours. After 24 hours, a 10-c.c. sample was extracted from each bottle and its oxygen content determined. The starting times for the bottles were arranged at 10-minute intervals, the 24-hour runs being so staggered as to allow sufficient time for the oxygen determination on each at the end of the run. Oxygen determinations were carried out on 10-c.c. samples from the container from which the bottles were filled, before, during and after the filling of the bottles.

The syringe-pipette method described by Whitney (1938) was followed for all oxygen determinations. From a series of preliminary readings it was judged that an experimental error of not more than 0·01 c.c. N/50 thiosulphate on the microburette reading was normally present, although about one reading in twenty showed a greater divergence from the mode (up to 0·04 c.c.) on the lower side. This is possibly due to occasional inefficiency of oxygen uptake by the manganous hydroxide. (The second control reading of Exp. 1, and the last reading of Exp. 3 (*infra*) are perhaps due to an error of this type, but have of course been included in the calculations). Oxygen introduced with the reagents ($MnCl_2$, NaOH–KI solutions) was calculated by blank experiments before and after the experimental runs; a 10-c.c. sample of distilled water, which had been boiled to expel air and cooled in the absence of air, was analysed by the normal technique, the reading representing oxygen introduced with the standard volumes of reagents. This factor is of importance only in calculating the absolute oxygen tension at the start of the experiment. Occasionally, during the induction of the sample into the barrel of the syringe, an air bubble entered past the plunger. All such experiments were rejected; their occurrence is noted to explain the discrepancy between the numbers of experimental and control readings in Exps. 1 and 3 (*infra*).

All beetles survived the treatment, being alive and healthy some days afterwards, although some showed temporary distress at the end of Exp. 1, where the final oxygen tension was very low.

The following results were obtained:

Experiment 1: 5–6/iii/41. Water temperature 20·5–21·0°C.

Initial oxygen concentration: 10-c.c. water sample contains equivalent of 0·28 c.c. 1·02N/50 sodium thiosulphate (four readings).

Oxygen introduced with reagents equivalent to 0·03 c.c. thiosulphate (four readings).

Whence initial oxygen content = 2·9 c.c. S.T.P./litre.

Oxygen concentration in controls, 24 hrs.: 10-c.c. samples contain equivalent of 0·28, 0·25, 0·28, 0·28 c.c. 1·02N/50 sodium thiosulphate.

Volume of bottle and number of adult *Coxelmis* included.	10-c.c. sample equivalent to x c.c. 1·02N/50 thiosulphate.	Oxygen consumption, c.c. S.T.P./beetle/hour.*
17·9 c.c. 15	0·13	$8·0 \times 10^{-5}$
15·0 c.c. 15	0·14	$6·2 \times 10^{-5}$
15·8 c.c. 15	0·15	$6·0 \times 10^{-5}$

Experiment 2: 6–7/iii/41. Water temperature 18·5–19·5°C.

Initial oxygen concentration: 10-c.c. water sample contains equivalent of 0·30, 0·29, 0·30, 0·31 c.c. 0·98N/50 sodium thiosulphate.

Oxygen introduced with reagents equivalent to 0·03 c.c. thiosulphate (two readings).

Whence initial oxygen content = 3·0 c.c., S.T.P./litre.

* Oxygen consumption, c.c. S.T.P./beetle/hour = 0·112 xvk/240n, where x = difference between microburette reading at end of experiment and average for controls, in c.c.; v = volume of bottle, in c.c.; k = reduction factor relating thiosulphate to N/50 strength; and n = number of beetles used.

Oxygen concentration in controls, 24 hrs.: 10-c.c. samples contain equivalent of 0·30, 0·29, 0·28 c.c. 0·98N/50 sodium thiosulphate.

Volume of bottle and number of adult *Coxelmis* included.	10-c.c. sample equivalent to x c.c. 0·98N/50 thiosulphate.	Oxygen consumption, c.c. S.T.P./beetle/hour.
17·9 c.c. 6	0·22	$9·6 \times 10^{-5}$
17·6 c.c. 6	0·24	$6·7 \times 10^{-5}$
17·2 c.c. 6	0·23	$7·9 \times 10^{-5}$

Experiment 3: 3–4/vi/41. Water temperature 14–16°C.

Initial oxygen concentration: 10-c.c. water sample contains equivalent of 0·36, 0·35, 0·35, 0·35, 0·37, 0·36, 0·36, 0·37, 0·36 c.c. N/42·3 sodium thiosulphate.

Oxygen introduced with reagents equivalent to 0·04 c.c. thiosulphate (three readings). Whence initial oxygen content = 4·2 c.c. S.T.P./litre.

Oxygen concentration in controls, 24 hrs.: 10-c.c. samples contain equivalent of 0·36, 0·37, 0·37, 0·36 c.c. N/42·3 sodium thiosulphate.

Volume of bottle and number of adult *Coxelmis* included.	10-c.c. sample equivalent to x c.c. N/42·3 thiosulphate.	Oxygen consumption, c.c. S.T.P./beetle/hour.
16·8 c.c. 8	0·32	$5·2 \times 10^{-5}$
16·8 c.c. 8	0·32	$5·2 \times 10^{-5}$
16·6 c.c. 8	0·32	$5·0 \times 10^{-5}$
16·2 c.c. 8	0·30	$7·3 \times 10^{-5}$
15·4 c.c. 8	0·27	$10·1 \times 10^{-5}$

The average individual oxygen consumption from these experiments is $7·0 \pm 1·7 \times 10^{-5}$ c.c. S.T.P./hour (11 determinations, in three sets differing in oxygen tension, temperature and season). Preliminary experiments using unsterilized stream water almost saturated with oxygen at its normal partial pressure in air gave somewhat higher readings (12 c.c. $\times 10^{-5}$ S.T.P. per hour for each beetle), but these experiments are not regarded as critical in view of the relatively high oxygen loss in the controls.

Taking the weight of living tissue in each individual as 1·0 mgm. (*supra*), the oxygen consumption of 7×10^{-5} c.c. S.T.P./hour is equivalent to 70 c.c. S.T.P./kgm./hr. This figure seems to be low in comparison with most insects; Ege (1918, p. 100) gives readings of 212–429 c.c. S.T.P./kgm./hr. for Dytiscidae, Notonectidae and Corixidae (resting specimens, in summer), but, for resting *Corixa* (op. cit., p. 102), 335 c.c. at 18°C. (summer) to 15·6 c.c. at 6°C. (winter). Chadwick and Gilmour (1940) give figures equivalent to 1,440 c.c. S.T.P./kgm./hr. for resting specimens of *Drosophila repleta* Wollaston, with an increase to 1,300% in flight, and these figures seem to be fairly representative for aerial insects.

It might be said that the low oxygen requirements of adults of *Coxelmis* are an adaptation to the aquatic environment, but it seems more reasonable to suppose that the relative inefficiency of oxygen uptake associated with continued submergence has led to a retardation of metabolism and activity. Pumping movements are never observed in submerged specimens, but are characteristic of beetles temporarily removed from water, when unusual activity always ensues. This is consistent with the view that submerged beetles are subject to chronic oxygen lack.

C. *Theoretical Considerations.*

It has been shown by the classic work of Ege (1918) that air stores of submerged respiring insects, in theory and practice, tend to decrease in volume, and finally, other things being equal, to disappear. This decrease follows from the very high invasion coefficient of carbon dioxide, and is only slightly delayed by the low invasion coefficient of nitrogen, which diffuses outwards in response to partial pressure differences. In insects such as *Coxelmis*, hydrofuge hairs are present, and meniscus formation between these will prevent continued reduction in actual volume of the air stores. Behind these menisci, lowered hydrostatic pressure of the air stores will ultimately counteract the tendency for further loss of nitrogen into solution, provided that the surface tension forces available are sufficient for the stresses placed upon them. It will be of interest to calculate the reduction in hydrostatic pressure of the air stores necessary to maintain the system in the steady state.

The following symbols may be adopted:

k = oxygen consumption of beetle, c.c. S.T.P./minute.

P = external pressure on beetle = 1 at.

P_i = pressure in gas store of beetle.

x = oxygen concentration %
n = nitrogen concentration %
c = carbon dioxide concentration %
} Average values for water-gas interface of air film; $x + n + c = 100$.

A = area of gas-water interface of beetle's air film, cm.^{2}.

γ_{O_2}, γ_{CO_2}, γ_{N_2} = invasion coefficients of oxygen, carbon dioxide and nitrogen, c.c. S.T.P./cm.^{2}/minute per atmosphere difference in partial pressure.

Two assumptions, likely to be, at best, near approximations, have been made: (1) That the R.Q. is unity, i.e., that k c.c. S.T.P. of carbon dioxide are produced per minute; (2) that the surrounding water is in equilibrium with a normal atmosphere, and remains so by circulation; the partial pressures of nitrogen, oxygen and carbon dioxide out of solution are thus taken as 0·7904P, 0·2093P and 0·0003P respectively. (It will be seen from the calculation below that the same results would be obtained regardless of the relative proportions, provided the sum of the partial pressures is and remains at P).

Then, in the steady state, $0{\cdot}7904P = \frac{nP_i}{100}$ .. .. (1)

$(0{\cdot}2093P - \frac{xP_i}{100})\ A\gamma_{O_2} = k$ .. .. (2)

$(\frac{cP_i}{100} - 0{\cdot}0003P)\ A\gamma_{CO_2} = k$ .. .. (3)

Also, $n + x + c = 100$ .. .. (4)

From (2), $\frac{k}{A\gamma_{O_2}} = 0{\cdot}0293P - \frac{xP_i}{100}$ .. .. (5)

From (3), $\frac{k}{A\gamma_{CO_2}} = \frac{cP_i}{100} - 0{\cdot}0003P$ .. .. (6)

From (1) and (4), $0{\cdot}7904P = \frac{(100 - x - c)P_i}{100} = P_i - (\frac{x + c}{100})\ P_i$ .. .. .. (7)

From (5) and (6), $\frac{k}{A}(\frac{1}{\gamma_{O_2}} - \frac{1}{\gamma_{CO_2}}) = 0{\cdot}2096P - \frac{(c + x)P_i}{100}$

$= 0{\cdot}2096P + 0{\cdot}7904P - P_i$, from 7, $= P - P_i$ .. .. (8)*

Substituting the values $k = 7{\cdot}0 \times 10^{-5}/60$ c.c. S.T.P./minute, A = 0·028 cm.^{2} and $\gamma_{O_2} = 0{\cdot}029$ c.c. S.T.P./cm.^{2}/minute/atmosphere,† and assuming $\frac{1}{\gamma_{CO_2}}$ tends to zero, we find the reduction in internal pressure theoretically required to maintain the respiration in the steady state = 0·0014 at. = 1·1 mm. Hg.

It has been noted that the distance between parallel adpressed hairs is of the order of 0·0025 mm. Semi-circular menisci of this order of size would cause a reduction in pressure of over half an atmosphere. If these hairs are, as they appear, really unwettable, then their spacing is far closer than is necessary for steady respiration of the order observed. The following experiments support this view:

(1). Adults of *Coxelmis novemnotata* were placed in water, which was then subjected to a decrease in pressure of 18 cm. Hg. until formation of air bubbles from solution and from the air stores had ceased. The pressure was then restored to normal

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* Footnote (added 24th November, 1941): If the respiratory quotient is not unity, but Q, then kQ c.c. S.T.P. of carbon dioxide are produced per minute by each individual, and the formulation becomes:

$$P - P_i = \frac{k}{A}(\frac{1}{\gamma_{O_2}} - \frac{Q}{\gamma_{CO_2}}).$$

For values of Q likely to apply (0·5-1·5), this is virtually the same expression as above, in view of the very high value of γ_{CO_2}.

† Value obtained experimentally by Ege (op. cit., p. 100) at 17°C., under conditions "which as nearly as possible correspond to the circumstances upon which they will be used".

and the beetles, kept continuously under the same water, were quickly examined under the binocular. Their air films were unchanged.

(2). This experiment was repeated, the reduced pressure being maintained for one hour after bubble formation had ceased. Under these circumstances the air stores would tend to come into equilibrium with water saturated at 18 cm. Hg. below atmospheric pressure. The air film was unchanged by the process.

(3). In none of the quantitative experiments described above was the air film dissolved, even at the end of Exp. 1, where the water was only about 20% saturated with oxygen.

OTHER SPECIES.

The adults of the following New South Wales species have the same general habits and field distribution as *Coxelmis novemnotata* (King): *Coxelmis trinotata* C. & Z., *Notriolus galstonius* C. & Z., *N. maculatus* (Cart.), and *Simsonia purpurea* (Cart.). In mixed cultures, the species of *Notriolus* die under conditions of oxygen lack before those of *Coxelmis*; this may possibly be due to their smaller area:mass ratio (length nearly 4 mm.).

In Tasmania the species *Simsonia tasmanica* (Blkb.) occurs not only in running water, but also on the littoral of highland lakes (e.g., Lake St. Clair). Although no determinations have been made, it is assumed that the waters of these lakes are well oxygenated.

In California the species *Helichus immsi* Hinton (sub-family Pelonominae) occurs commonly in shallow pools beside running creeks (e.g., Niles Canyon). General observations suggest that, although it possesses hydrofuge hairs and an air film as in *Coxelmis*, it does not depend on this for continuous oxygen exchange. Its greater average length (8 mm.) and weight (20 mgm.) render its area:mass ratio low (about one-third that of *Coxelmis* spp.), and it is more active, leaving the water readily. Individuals placed on a piece of log in cool air-free stream water immediately and rapidly made their way to the surface, remaining at or just above it and returning there if submerged again. No quantitative data on the oxygen consumption of this species are available, but appreciable uptake of oxygen by unsaturated water from the beetles' air stores was recorded after three hours' immersion. This fact should be considered in assessing the reliability of the quantitative experiments on *Coxelmis* noted above; the values calculated are all likely to err on the lower side for this reason.

From the work of Hinton (1936), and papers quoted therein, it would appear that the European species *Dryops luridus* Erichson is far less obligate-aquatic than the Australian genera here discussed.

SUMMARY.

1. Larvae and adults of *Coxelmis novemnotata* (King) are aquatic, and their oxygen requirements are satisfied from oxygen in solution. The larvae do not seem to differ from other aquatic insect larvae with closed tracheal systems.

2. Continuous oxygen intake is carried out in the adult by means of a ventral gas film which acts as a gill; average oxygen consumption appears to be of the order of 7×10^{-5} c.c. S.T.P./hour for each individual, representing 70 c.c. S.T.P./kgm. of living tissue/hour.

3. The hydrofuge hairs of the ventral surface of the adult body prevent the decrease in volume of air stores normally inherent in subaquatic respiration in insects.

4. These hairs are so spaced that the surface tension forces of the menisci between them could produce considerably greater lowering of internal pressure than is theoretically required to maintain the system in the steady state.

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