Comp. Biochem. Physiol., 1974, Vol. 47A, pp. 17 to 22. Pergamon Press. Printed in Great Britain

EFFECTS OF HYPEROXIA ON SURVIVAL OF BENTHIC MARINE INVERTEBRATES*

J. J. TORRES and C. P. MANGUM

Department of Biology, College of William and Mary, Williamsburg, Virginia 23185, U.S.A.

(*Received* 13 *March* 1973)

Abstract—1. The effects of oxygen levels considerably in excess of air saturation on the survival of fourteen species of marine invertebrates were tested.

2. The anemones *Diadumene leucolena* and *Haliplanella luciae* and the clam *Mya arenaria* suffered high mortality.

3. Members of other species, including three hemoprotein-containing annelids, were not killed by prolonged exposure to hyperoxia. These results do not support the hypothesis that high oxygen affinity hemoglobins serve to buffer the tissues from otherwise toxic hyperoxia.

INTRODUCTION

Fox & Taylor (1955) presented evidence that oxygen levels equalling and exceeding air saturation of natural waters are toxic to bottom-dwelling aquatic invertebrates. From an adaptive standpoint, this finding on lacustrine species that inhabit the oxygen-poor hypolimnion is not surprising; and indeed, oxygen poisoning of tubificid oligochaetes has been recently confirmed (Walker, 1970). But Fox & Taylor (1955) also reported toxic effects of air-saturated conditions on intertidal marine species such as the orbiniid polychaete *Scoloplos armiger*. This finding is surprising, since most benthic polychaetes are believed to generate a relatively high oxygen microhabitat by vigorous muscular ventilation of their tubes or burrows (Mangum, 1973).

Moreover, their results have been cited in support of the "oxygen buffer" theory of high oxygen affinity hemoglobins in benthic annelids (Manwell, 1963). According to this notion, if an animal's blood pigment is completely oxygenated at a relatively low environmental pO_2, then tissue pO_2 never reaches the high levels that become toxic. The hypothesis also requires the condition that most of the oxygen consumed by tissues is supplied by the blood pigment, which is probably not true of marine invertebrates, as long as normal ventilation is not prevented by low tide (Mangum, 1970).

Nonetheless, it is important to know whether high oxygen toxicity accompanies the presence of high oxygen affinity respiratory pigments in animals that are not restricted to essentially anoxic habitats. At least one alternative factor whose

* Supported by N.S.F. Research Grants Nos. GB-20335 and GA-34221.

toxic effects were unknown at the time of Fox & Taylor's (1955) experiments might explain their results. In the course of hemoprotein synthesis, benthic annelids make extremely large excess quantities of several different porphyrin compounds, which render them highly susceptible to the toxic effects of near u.v. light (Mangum & Dales, 1965). While their porphyria is not a problem in the normal darkness of their tubes and burrows, it is a problem under laboratory conditions unless they are carefully screened from prolonged exposure to wavelengths corresponding to the Sorêt band.

MATERIALS AND METHODS

The anemones *Haloclava producta* and *Metridium senile* were collected at Woods Hole, Massachusetts, and maintained in running sea water (salinity 31–32‰). The bloodworm *Glycera dibranchiata* was purchased from a coin-operated machine at a local bait store and maintained in recirculating aerated aquaria for a minimum of 2 days prior to experimentation. All other species were collected from the York (18–21‰) or James (2–7‰) River estuaries in Virginia and maintained in natural water of the appropriate salinity. The annelids and the burrowing anemone *H. producta* were kept in short glass tubes corresponding to their body length during the course of exposure.

In several preliminary experiments oxygen exposure chambers consisted of large airtight vessels which were flushed every 24 hr with pure oxygen (experimental) or air (control). A large volume of water prevented appreciable change in the dissolved oxygen content by the animals' respiratory activities. Mixing within the containers was achieved by either a magnetic stirring bar or variable speed rotating platform. Results from these experiments are designated with an asterisk in Table 1.

TABLE 1—EFFECT OF EXPOSURE TO HYPERBARIC OXYGEN

Species	Oxygen concentration (ppm)	Temperature (°C)	No. of animals		Exposure period (days)
			Start	End	
Coelenterata					
Scyphozoa					
A. aurita					
Experimental	20·0–27·2	15–18·5	50	50	7–9
Control	8·0–9·0	15–18·5	50	50	7–9
Anthozoa					
*D. leucolena**					
Experimental	> 20·0	15–18	10	1	7–9
Control	7·8–9·0	15–18	10	10	7–9
H. luciae					
Experimental	28·9 ± 5·5 (S.E.) (24·0–35·1)	20·0	23	2	11
Control	9·2 ± 0·2 (S.E.) (7·3–9·4)	20·0	23	21	11
H. producta					
Experimental	23·0	10·0	4	4	7
Control	8·7	10·0	4	4	7
M. senile					
Experimental	23·0	10·0	3	3	5
Control	8·7	10·0	3	3	5

TABLE 1 (*cont.*)

Species	Oxygen concentration (ppm)	Temperature (°C)	No. of animals		Exposure period (days)
			Start	End	
Annelida					
Polychaeta					
G. dibranchiata					
Experimental	20·0–29·1	10–24	12	9	7–13
Control	7·3–9·1	10–24	19	13	7–13
N. succinea					
Experimental	21·0–36·1	13–25	17	13	3–14
Control	5·7–9·4	13–25	17	13	3–14
*P. ligni**					
Experimental	> 20·0	16·5	4	3	28
Control	7·8–9·0	16·5	4	3	28
Mollusca					
Gastropoda					
*Nassarius obsoletus**					
Experimental	> 20·0	22·0	10	10	7
Control	7·2	22·0	10	10	7
Lamellibranchia					
M. demissus					
Experimental	26·9–35·1	15–19·5	4	4	7–9
Control	7·8–9·2	15–19·5	4	4	7–9
M. arenaria					
Experimental	20·0	19·5	6	0	9
Control	8·0	19·5	6	6	9
R. cuneata					
Experimental	> 20·0	19·5	4	4	9
Control	6·0–8·0	19·5	4	4	9
Arthropoda					
Crustacea					
R. harrisi					
Experimental	20·0	18·3	3	2	7
Control	8·0	18·3	3	2	7
Urochordata					
M. manhattensis					
Experimental	21·8–40·4	16–25	7	7	8–14
Control	7·6–9·3	16–25	7	7	8–14

In order to maintain more stable levels, airtight chambers in subsequent experiments were flushed continuously with pure oxygen or air. Flow was stopped only to remove dead animals or to measure oxygen and pH, which varied from 8·2 to 8·6 in experimental chambers and 7·6–8·0 in controls. Oxygen levels were checked periodically by either Winkler titrations or measurement with the pO_2 electrode (Type E-5046) of a Radiometer blood gas apparatus (BMS1). It varied only 0·2–0·5 ppm/day. The Radiometer apparatus was also used to measure small samples withdrawn from the worm tubes and coelomic cavities.

All experiments were conducted in darkness. Although the temperature varied for different sets of experiments, it was held constant during each individual experiment.

At the termination of each experiment, the animals were removed from the chambers and placed in aerated aquaria. They were then observed for a minimum of 2 days to distinguish post-exposure recovery from lethal responses.

RESULTS

Two species of the four anthozoans tested exhibited a negative response (see Table 1). *Haliplanella luciae* and *Diadumene leucolena*, after 3 days of exposure, lost the ability to set and flower. Continued exposure caused the animals to coat themselves with mucus and darken to a brownish hue. In outward appearance experimental animals closely resembled the over-wintering encysted form (Sassaman & Mangum, 1970). No recovery was observed on the part of *D. leucolena*. Two of twenty-three *H. luciae* survived, but they were shrunken in appearance and their ability to set was impaired. Control animals were outwardly healthy, well set and in full flower. The two other anemones, *Metridium senile* and *Haloclava producta*, showed no ill effects whatsoever, during or after the experiment. The polyp form of the scyphozoan jellyfish, *Aurelia aurita*, did not exhibit gross morphological symptoms of oxygen poisoning and did not suffer any mortality. However, only a very small number (10 per cent) of the experimental organisms set, whereas 80 per cent set in the control vessel.

Neither the clam, *Rangia cuneata*, nor the mussel, *Modiolus demissus*, was visibly affected by hyperoxia. Normal ventilation was suggested by the observation of extended siphons. The former result is particularly interesting because of the notable adaptations of the species for anaerobiosis (Chen & Awapara, 1969). Young *Mya arenaria* with a shell size of roughly 5 mm suffered total mortality. The shells of many of the animals disintegrated, and all failed to revive when placed in fresh York River water. The common sea squirt, *Molgula manhattanensis*, remained healthy during and after exposure, which was indicated by fully extended siphons, retractile on touch. The sand crab, *Rhithropanopeus harrisi*, did not appear to be affected by hyperoxia. The deaths of one each of the experimental and control animals were probably due to aggression, and were followed by cannibalization.

The hemoprotein-containing polychaetes, *Nereis succinea*, *Polydora ligni* and *Glycera dibranchiata*, exhibited no negative responses to hyperoxia above those in controls. However, mortality in both experimental and control vessels was higher than anticipated. In *G. dibranchiata*, an initial experiment on six animals was terminated after 7 days with only one survivor in the control vessels. To test the possible detrimental effects of oxygen levels approximating air-saturated conditions, also a part of Fox & Taylor's (1955) hypothesis, we investigated survival at lower oxygen levels. Seven to fifteen worms exposed to each of three pO_2's below 159 mm Hg (24, 49 and 82% air saturation), for periods varying from 2 to 7 days, suffered no mortality. Three of fourteen worms died after exposure to 2·8 mm Hg for 24 hr, a result consistent with our earlier report of a 3-day lethal

limit in response to anoxia (Hoffman & Mangum, 1970). Therefore, we believe that the mortality in both control and experimental phases of our experiments on annelids may be due to delayed effects of a temperature increase prior to the experiment. These determinations were carried out during the colder months of the year, and we pre-exposed the animals to the experimental temperature for 1 week.

We noted a peculiar secondary effect in the annelids, however. Tissues contiguous with hemoprotein-containing fluids in all three species became bright red within 48 hr of exposure to high oxygen conditions, particularly when observed next to the control animals. We attempted to measure hemoglobin concentration in *N. succinea* and *G. dibranchiata*, but the large variance in both experimental and control worms, which was reported previously for *G. dibranchiata* (Hoffmann & Mangum, 1970), prevented meaningful comparison. Since *G. dibranchiata* hemoglobin is 95 per cent oxygenated at external air saturation (Mangum & Carhart, 1972), the increased redness of the experimental animals cannot be explained by an increased percentage of oxyhemoglobin. Although increased synthesis of hemoglobin by animals exposed to low oxygen levels was demonstrated by Fox (1955), a similar effect of hyperbaric oxygen is not known.

The pO_2 of coelomic fluid of four experimental bloodworms (*G. dibranchiata*) equilibrated to a tube pO_2 of 603·5 mm Hg is 184 ± 17 (S.E.) mm Hg. In contrast, the control value for nine worms equilibrated to a tube pO_2 of 159 mm Hg is 86·7 ± 2·4 (S.E.) mm Hg. This result confirms that the tissues experienced a pO_2 well above normal with no resultant injury.

DISCUSSION

The results of this experiment suggest that oxygen poisoning is not a widespread phenomenon among benthic marine invertebrates. The three species of mud-dwelling polychaetes which were used in this experiment are especially interesting because they contain blood hemoproteins, at least one of which is a high oxygen affinity hemoglobin (Hoffmann & Mangum, 1970). Unlike the animals tested by Fox & Taylor (1955), survival of these three species is not detectably oxygen dependent. This result does not support the hypothesis that animals found in benthic environments characterized by fluctuating oxygen levels are detrimentally affected by high internal pO_2's. Similarly, an oxygen buffer function for their hemoglobins does not seem plausible.

It is curious that two out of the three species which were affected by hyperoxia are found in water which is normally very close to air saturation. Both *H. luciae* and *D. leucolena* reach maximum density in such places as bridge pilings, where the current is quite swift (Sassaman & Mangum, 1970).

REFERENCES

CHEN C. & AWAPARA J. (1969) Effect of oxygen on the end-products of glycolysis in *Rangia cuneata*. *Comp. Biochem. Physiol.* **31**, 395–402.

FOX H. M. (1955) The effect of oxygen on the concentration of haem in invertebrates. *Proc. R. Soc. Lond. B* **143**, 203–214.

FOX H. M. & TAYLOR A. E. R. (1955) Tolerance of oxygen by aquatic invertebrates. *Proc. R. Soc. Lond. B* **143**, 214–225.

HOFFMANN R. J. & MANGUM C. P. (1970) The function of coelomic cell hemoglobin in the polychaete *Glycera dibranchiata*. *Comp. Biochem. Physiol.* **36**, 211–228.

MANGUM C. P. (1970) Respiratory physiology in annelids. *Am. Sci.* **58**, 641–647.

MANGUM C. P. (1973) Evaluation of the functional properties of invertebrate hemoglobins. *Neth. J. Sea Res.* Vol. **7**. (In press.)

MANGUM C. P. & CARHART J. A. (1972) Oxygen equilibrium of coelomic cell hemoglobin from the bloodworm *Glycera dibranchiata*. *Comp. Biochem. Physiol.* **43A**, 949–957.

MANGUM C. P. & DALES R. P. (1965) Products of haem synthesis in polychaetes. *Comp. Biochem. Physiol.* **15**, 237–257.

MANWELL C. (1963) Chemistry, genetics, and function of invertebrate respiratory pigments—configurational changes and allosteric effects. In *Oxygen in the Animal Organism* (Edited by DICKENS F. & NEIL E.), pp. 49–120. MacMillan, New York.

SASSAMAN C. & MANGUM C. P. (1970) Patterns of temperature adaptation in North American coastal actinians. *Mar. Biol.* **7**, 123–130.

WALKER J. G. (1970) Oxygen poisoning in the annelid *Tubifex tubifex*—I. Response to oxygen exposure. *Biol. Bull., mar. biol. Lab., Woods Hole* **138**, 235–244.

Key Word Index—Hyperoxia; oxygen; invertebrates; anemones.