Chapter II.9 Bacterial Growth and Antibiotics in Animal Respirometry

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1 Introduction

The uncontrolled contribution of bacterial oxygen consumption in animal respirometry respresents a substantial problem and results in an ambiguous reading of the animal's metabolic rate. This problem became especially important when the application of polarographic oxygen sensors (POS) made possible long-term measurements of the dynamics of an animal's energy metabolism (which may be superimposed by bacterial growth). Bacterial growth is most rapid on free surfaces [42, 60] such as the inner walls of the animal chamber, but also in the stirring chamber of the POS, valves, connecting tubings, etc.

Some researchers resort to chemical bactericides to eliminate or inhibit the growth of bacteria in the system [1, 4, 9, 32], whereas others determine the bacterial oxygen consumption in a blank experiment and subtract it from the observed total oxygen uptake ([21, 24, 51], Chap. II.3). However, an inspection of the literature suggests that many workers have ignored the part played by bacterial action in oxygen consumption measurements.

2 Antibiotics

The range of application of antibiotics is very wide, and during the past decades new types have been continuously developed, while at the same time significant progress has been made in clarifying their mechanisms of action [23, 25]. Antibiotics have been used in respirometry in the belief that they exert no influence on the oxygen consumption of the experimental animals [18, 32]. It also should be noted that considerable differences exist in the chemical properties of various antibiotics. Their stability and antimicrobial activity depends to a large extent on the pH and temperature of the medium. Hence the physical and chemical properties of the experimental media largely determine the choice of antibiotic. The properties of some antibiotics have been compiled from the literature in Tables 1 and 2 [12, 20, 23, 26, 29, 41, 46, 54]. Not every antibiotic is suitable for a particular experiment; for instance under anaerobic conditions kanamycin, neomycin and streptomycin are inactive.

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Polarographic Oxygen Sensors (ed. by Gnaiger/Forstner)

The mechanism of action also determines the applicability of a specific antibiotic in respirometry. The following classification is given [41]:

a) Antibiotics interfering with nucleic acid or protein biosynthesis: actinomycins, adriamycin, amikacin, aminoglycosides, bleomycins, capreomycin, chloramphenicol, clindamycin, fusidic acid, lincomycin, macrolide antibiotics, mitomycins, puromycin, rifamycins, sisomicin, streptonigrin, streptozotocin, thiamphenicol, viomycin.
b) Antibiotics affecting the function of the cytoplasmatic membrane: amphotericin B, candicidin, gramicidins, nystatin, pimaricin, polymyxins, streptomycin, trichomycin, tyrocidins.
c) Antibiotics interfering with cell wall biosynthesis: bacitracin, D-cycloserine, cephalosporins, fosfomycin, novobiocin, penicillins, ristocetins, vancomycin.

Due to their unspecific attack on cell metabolism, the first two classes not only damage bacteria, but also disturb the metabolism of the experimental animals. The third class of antibiotics specifically inhibits cell wall synthesis in the bacteria and so hardly influences animal cells.

In selecting an antibiotic it is also important to know the antimicrobial spectra of the antibiotics (Table 2). They can be distinguished as follows [54]:

a) Wide-spectrum antibiotics: ampicillin, cephalosporins, chloramphenicol, rifampicin, tetracyclines.
b) Antibiotics preferentially effective against gram-negative bacteria: gentamicin, kanamycins, neomycins, paromomycin, polymyxin B and E, streptomycin.
c) Antibiotics preferentially effective against gram-positive bacteria: bacitracin, erythromycines, fusidic acid, lincomycin, novobiocin, penicillins, peptolid-antibiotics, rifomycin, ristocetin, vancomycin.
d) Fungicids: amphotericin B, griseofulvin, nystatin, trichomycin.
e) Antibiotics inhibiting protozoa: fumagillin, trichomycin.

For use in seawater, antibiotics effective against gram-negative bacteria are to be preferred. Penicillin is inapplicable, since it inhibits mucopeptide synthesis and is thus detrimental to gram-positive bacteria only.

Antibiotics can be classified according to their undesirable toxicity to animals [41]:

a) Antibiotics with low toxicity: cephalosporins, erythromycin, fusidic acid, lincomycins, penicillins, thiamphenicol.
b) Toxic antibiotics causing reversible and irreversible damage: aminoglycoside antibiotics (e.g. neomycin), amphotericin B, chloramphenicol, novobiocin, polymyxins, viomycin, paromomycin, tyrothricin, nystatin.

Table 1. Properties of some antibiotics: solubility in water, optimal pH range for maximum antimicrobial activity, inactivation rate (%) and inactivating agents in aqueous solutions, and cross-resistance (referring to antibiotic No.)

Antibiotic	Solubility mg cm^{-3}	pH	Inactivation rate pH	°C	days	%	Inactivating agents	Cross-resistance
1. Amphomycin	Soluble	4.5–7.5	7	24–25	30	Stable		
2. Ampicillin	Acid: 100	5.5	7	24	7	20		[32]
3. Bacitracin	1000	6.5 (6–7.5)	6	5	14	Stable	pH ⩾ 9; high saline concentrations cause precipitation; sodium thiosulfate; ions of heavy metals Cd^{2+}, Mn^{2+}, Zn^{2+}; H_2O_2;	
					2 h	100		
4. Candicidin			7	4	7	Stable		
5. Carbenicillin	Good		2	21	2	50		[2]
6. Carbomycin			5–7	25	11	Stable		[16]
7. Cephaloridin	220–250	7.5–8.5		4	4	Stable	pH > 8; light	[8]
				25	1–4	Stable		
8. Cephalothin	200–300	6.5 (6–7.3)		4	2	Stable		[7, 18]
				37	12 h	11–23		
				37	24 h	36–39		
				37	48 h	47–55		
9. Chloramphenicol	2.5–4.4	7.4–8.0	2.5–9	24	1	Stable	pH > 9.5; phenylalanine; cyclic amino-acids; metal ions do not deactivate chloramphenicol	[40]
			7	30	60	Stable		
			7	5	700	Stable		
10. Chlortetracycline	Base: 0.55	6.1–6.6	7	37	10 h	65	Bi- and trivalent metallic cations	[30, 39]
	8.6		7	37	24 h	95		
			8	37	10 h	92		
			8	37	24 h	99.7		
			8.5		4–5 h	50		
11. Cloxacillin	Good			4	7	5		
				25	4	15		
12. Cycloserine	100	6.4–7.4	7	37	7	25	D-alanine; Mycobyctin;	
			7	37	14	38		
13. Colistin	> 1	6.5–7.5	6	30	14	Stable	pH > 6; Ca^{2+}	[35]
			7	30	3	10		
			7	30	16	57		
			9		1	50		
14. Dicloxacillin	150			24	2	Stable		[11, 24, 29, 32–34]
				24	9	70		
				4	14	10		
15. Dihydro-streptomycin	250–500	7.5–8	4–7	4	90	Stable	More stable than streptomycin; not deactivated by cysteine	[39]
			4–7	28	60	Stable		
16. Erythromycin	Base: 2.1	8–8.5	6		1–3 h	100	Acids; pH < 6; anaerobiosis does not modify activity; not affected by metallic ions	[6, 28]
	10–20–200		7	25	1	14		
			8.5	–25–+4	56	Stable		
17. Framycetin	Soluble		2–9	25	30	Stable		[25]
18. Fusidic acid	Alkali-salt	6			36 h	15	pH > 7	
19. Gabromycin	High	7.4–8.0					pH < 6; proteins and anaerobiosis do not inhibit the activity	
20. Gentamycin	Sulphate: > 20	7.8	2.2–10			Stable	Na-, K-, Ca-salts; CO_3^{2-}, SO_4^{2-}, Cl^-, PO_4^{3-}, NO_3^-	[15, 22, 25, 31]
21. Griseofulvin	0.01		3–8.8			Stable	Purine	
22. Kanamycin	Sulphate: 360	7.6–8.0	2.6–7.9	25	180	1.57	Phosphate, citrate, chloride, Mg^{2+}, anaerobiosis, CO_2^-, K^+, Ni^{2+}, Fe^{2+}, PO_4^-, Mg^{2+}	[15, 25, 31, 39]
			2.6–7.9	37	180	1.9		
			2.6–7.9	45	120	2.8		
23. Lincomycin	Base: 500	8–8.5		70	180	Stable	Erythromycine	
24. Methicillin	Good		7–7.2	4	5	20	pH 4–5; kanamycin; streptomycin	[11, 14, 29, 32–34]
			7–7.2	25	5	50		
			7–7.2	37	2.5	50		

Table 1 (continued)

Antibiotic	Solubility mg cm^{-3}	pH	Inactivation pH	°C	days	%	Inactivating agents	Cross-resistance
25. Neomycin B	6.3	7.4–8.0	8	25	360	Stable	Anaerobiosis, RNA, glucose, Cl^-, Na^+, K^+, PO_4^{3-}, $Fe^{2+,3+}$, Mg^{2-}, Aluminium ions	[14, 15, 32–34, 39]
26. Nitrofurantion	0.21	5.5–6.0	7	37	1	Stable	pH > 9, < 4	
27. Novobiocin	100	5.5	7–10	24	60	50	Bivalent metallic cations	
28. Oleandomycin	Base: 5 Phosphate: > 1000	8.0	2.2–9 5–7 9	37 24 25	1 1 21	Stable Stable Stable		[16]
29. Oxacillin	Good	6–6.6		4 24 24	15 7 15	6 9 38		[11, 14, 24, 32–34]
30. Oxytetracyclin	Base: 0.6 6.9	6.1–6.6	7 7 8 8	37 37 37 37	10 h 1 10 h 1	34 66 25 75	Bi- and trivalent metallic cations	[10, 40]
31. Paromomycin	Good	7.6–8.0						
32. Penicillin G	> 20	6–6.5	6.5 6.5	4 24–25	3–7 2	Stable 50	pH < 4, pH > 8, Pb, Cu, Hg, Zn, Sn, Cd, some amino acids, H_2O_2, rubber, glycerine, ethyl alcohol	[33, 34]
33. Penicillin V	> 750		5	37	33 h	50		[32, 34]
34. Pheneticillin	Good		6.5	4–35		Stable		[32, 33]
35. Polymyxin B	25	6.5–7.5	6–7	38	360	Stable	pH 2, 8; bivalent cations;	[13]
36. Propicillin	Good		6	24	7	12		[34]
37. Rifomycin	5%	6.0–7.4	7	25	700	Stable		

38. Spectinomycin	225	> 8.0	8	30	50	50		
39. Streptomycin	250–500	7.5–8.0	4–7 4–7 7	28 4 15	60 90 30	Stable Stable Stable	pH < 2; pH > 8.5; $t > 28°C$; $KMnO_4$; KJO_4; H_2O_2, HNO_3, ascorbic acid, glucose, NO_3^-, OCN^-, Mg, Ca, DNA, urea, anaerobiosis	[15]
40. Tetracycline	Base: 1.7 10.9	6.1–6.6	7 7 8 8	37 37 37 37	10 h 24 h 10 h 24 h	2 42 36 82	Bi- and trivalent cations	[10, 30]
41. Vancomycin	> 100	8.0	3–7	37	6	10		
42. Viomycin	5.6–7.8	7.4–8.4	5–6	24	7	Stable	pH > 9	[22, 39]

Table 2. Antimicrobial spectra of some antibiotics (+ efficient, ± poorly efficient, – inefficient against gram-positive or gram-negative bacteria), minimal inhibitory concentrations and development of resistance (for bacteria)

Antibiotic	Bacteria				Fungi	Protozoa	Resistance
	G^+	G^-	G^+	G^-			
			$\mu g\ cm^{-3}$		$\mu g\ cm^{-3}$	$\mu g\ cm^{-3}$	
Adicillin	±	±	5–10	1.5			
Amphomycin	+	–	2.5–6.2		Resistant	Resistant	
Amphotericin B	–	–			0.04–3.7	Nearly all res.	None
Ampicillin	+	±	0.01–1.5	>8			
Bacitracin	+	–	0.07–27	–10000	Resistant	Nearly all res.	Slow
Boromycin	+	–	0.05		Active		
Candicidin	–	–			0.5–50		
Capreomycin	+	–	0.5–20				Rapid
Carbenicillin	+	±	0.2–1.56	250			
Carbomycin	+	–	2–5		Resistant	30–250	
Cephalexin	+	±	50	50			
Cephaloridin	+	±	0.01–5	0.1–100	Resistant	Resistant	Slow
Cephalothin	+	±	0.02–8	>100		Resistant	Slow
Chloramphenicol	+	+	0.5–20	0.4–30	Resistant	125–2000	Slow
Chlortetracycline	+	+	0.3–20	0.1–50	Resistant	25–1000	Moderate-fast
Cloxacillin	+	–					
Colistin	–	+		0.1–50	20	125	Slow
Cycloserin	±	+	6.5–50	<125			Slow
Demethyl-chlortetracycline	+	+	0.3–20	0.1–50	Resistant	Nearly all res.	Moderate-fast
Dicloxacillin	+	–					
Dihydrostrepto-mycin	±	+	1–25	0.5–25	Resistant	Resistant	Rapid
Erythromycin	+	–	0.01–2		Resistant	Nearly all res.	Rapid
Framycetin	+	+					Slow
Fumagillin	+	–			Resistant	Effective	
Fusidic acid	+	–	0.2–10		Resistant	Resistant	Rapid
Gabromycin	+	+	2–7	1–5			None-slow
Gentamycin	+	+	0.8–40	1–50	>100–>1000	Resistant	Moderate-fast
Gramicidin	+	–	1–10				
Griseofulvin					0.2–15		None
Hamycin	–	–			0.01–4		
Kanamycin	±	+	4–500	0.5–15	Resistant	Nearly all res.	Moderate-fast
Kitasamycin	+	–	0.2–3		Resistant		
Lincomycin	+	–	0.04–32	>200	Resistant		Rapid
Myxin	+	+	0.6–2.5	0.4–3.2	1–10		
Nactins	+	–	1.0		10		
Neomycin B	±	+	0.5–150	0.5–15	Resistant	40–3000	Moderate-fast
Nitrofurantoin	+	+	2–3	0.002–20			Slow
Novobiocin	+	+	0.1–6	2–25	10–1000	125	Rapid
Nystatin	–	–			0.6–5–10–30	250	None
Oleandomycin	+	–	0.5–3				Rapid
Oxacillin	+	–	0.06–1.5				
Oxytetracycline	+	–	0.3–20	0.1–50	Resistant	30–250	Moderate-fast

Table 2 (continued)

Antibiotic	Bacteria				Fungi	Protozoa	Resistance
	G^+	G^-	G^+	G^-			
			$\mu g\ cm^{-3}$		$\mu g\ cm^{-3}$	$\mu g\ cm^{-3}$	
Paramomycin	–	+		0.8–25	Resistant	Nearly all res.	Moderate-fast
Penicillin G	+	±	0.006–3	>100	Resistant	Resistant	Slow
Penicillin V	+	–	0.01–8				Slow
Peptolid-antibiotics	+	–	0.1–4		Resistant	Resistant	Moderate-fast
Phenethicillin	+	±	0.03–4	>100			
Pimaricin					1–12	Nearly all res.	
Polymixin B	–	+		0.05–5	125–250	125	Slow
Propicillin	+	±	0.03–33				
Rifamid	+	±	0.03–10	25–250			Rapid
Rifampicin	+	±	0.01–0.1	1–50	>100		Rapid
Rifamycin	+	±	0.03–0.1 (10)	25–250			Rapid
Ristocetin	+	–	0.5–4		Resistant	Resistant	None
Rolitetracycline	+	+	0.3–20	0.1–50	Resistant	Nearly all res.	Moderate fast
Spectinomycin	–	+		4–500	Resistant	Resistant	Rapid
Spiramycin	+	–	0.1–5				Moderate-fast
Streptomycin	±	–	1–25	0.5–25	Resistant	Resistant	Rapid
Tetracycline	+	+	0.3–20	0.1–50	Resistant	60–250	Moderate-fast
Trichomycin					0.6–10	250	None
Tyrothricin	+	–	1–10				None-slow
Vancomycin	+	–	0.5–3		Resistant	Resistant	None
Viomycin	–	+		2–12			Rapid

Table 3 gives examples of the effect of individual antibiotics and their combinations on various aquatic animals. Penicillin has the lowest toxicity, which makes possible its use even in high doses. Kanamycin, neomycin, polymyxins and streptomycin may lead to a neuromuscular block. Kanamycin is less toxic than neomycin and paromomycin, but should not be used in combination with either of these since this leads to a considerable increase in toxicity. A combination of streptomycin and dihydrostreptomycin reduces the toxicity of the individual components. Of the tetracyclines, tetracycline, followed by oxytetracycline, combine optimal antibiotic properties, i.e., low toxicity and a high bactericidal effect. Streptomycin, chloramphenicol, kanamycin, and neomycin are antibiotics with long half-lives in seawater, but due to their high toxicity to invertebrates, their dosage may be critical [12]. Auromycin, terramycin, and polymyxin B are effective against marine bacteria but are toxic to phytoplankton, while penicillin, kanamycin, neomycin, and streptomycin are suitable for phytoplankton cultures [3, 47]. Chlortetracycline, oxytetracycline, bacitracin, carbomycin, Oleandomycin, tetracycline, erythromycin and penicillins have short half-lives in solution, and are therefore unsuitable for long-term experiments [12]. Antibiotics may not only disturb oxygen uptake in animals but also induce anoxic contributions to the total metabolic rate [19].

Table 3. Effect of antibiotics on physiological parameters in animals

Genera	Stage[a]	Antibiotics[b]	μg cm^{-3}	Days[c]	Parameter	Effect[d]	Ref.
Coelenterata							
Tubularia	a	PEN + STR	100 + 100	1	Regeneration	+	[17]
Tubularia	a	PEN + STR	400 + 400	1	Regeneration	+	[17]
Nemertina							
Amphiporus	a	STR + DISTR	750–5000	10	Regeneration	0	[52]
Cerebratulus	a	STR + DISTR	750–5000	10	Regeneration	0	[52]
Lineus	a	STR + DISTR	750–5000	10	Regeneration	0	[52]
Annelida							
Cognettia	a	FRA	250	4	Growth	+	[28]
Cognettia	a	PEN	250	4	Growth	+	[28]
Cognettia	a	STR	250	4	Growth	+	[28]
Lumbriculus	a	STR + NEO	20–200		Heat dissipation	+	[19]
Mollusca							
Adula	l	PENG + STR	50 + 100	5	Survival	0	[39]
Australorbis	l	PENG + STR	60*+ 100	3–4	Growth	–	[8]
Australorbis	l	STR	10	3–4	Growth	0	[8]
Australorbis	l	PENG	60*	3–4	Growth	0	[8]
Mya	l	PEN + STR	30*+ 100		Growth	0	[49]
Ostrea	l	PENG	30*	7	Yield of spat	+	[53]
Ostrea	l	PENG	30*	7	Growth	0	[53]
Ostrea	l	PENG + STR	30*+ 50	7	Growth	–	[53]
Tritona	l	STR + PENG	50 + 60	24	Growth	–	[27]
Tritona	l	STR + PENG	50 + 60	24	Survival	+	[27]
Crustacea							
Artemia	l + a	PENG	150	37	Growth	0	[12]
Artemia	l + a	PENG	200	7	Growth	–	[12]
Artemia	l	STR	200	37	Growth	0	[12]
Artemia	l + a	STR	300	7	Growth	–	[12]
Artemia	a	STR	250	37	Growth	0	[12]
Artemia	l	POL	30	37	Growth	0	[12]
Artemia	l + a	POL	50	7	Growth	–	[12]
Artemia	a	POL	40	37	Growth	0	[12]
Artemia	l + a	CHL	25	37	Growth	0	[12]
Artemia	l + a	CHL	50	7	Growth	–	[12]
Artemia	l + a	NAR	10	37	Growth	0	[12]
Artemia	l	NAR	15	7	Growth	–	[12]
Artemia	a	NAR	20	7	Growth	–	[12]
Artemia	l + a	CAN	2.5	37	Growth	0	[12]
Artemia	l	CAN	3	7	Growth	–	[12]
Artemia	a	CAN	5	7	Growth	–	[12]
Artemia	l + a	NYS	10	37	Growth	0	[12]
Artemia	l	NYS	15	7	Growth	–	[12]
Artemia	a	NYS	30	7	Growth	–	[12]
Artemia	l + a	KAN	250	37	Growth	0	[12]
Artemia	l	KAN	500	7	Growth	–	[12]
Artemia	a	KAN	750	7	Growth	–	[12]
Artemia	l + a	NEO	5	37	Growth	0	[12]
Artemia	l	NEO	10	7	Growth	–	[12]
Artemia	a	NEO	15	7	Growth	–	[12]

Table 3 (continued)

Genera	Stage[a]	Antibiotics[b]	μg cm^{-3}	Days[c]	Parameter	Effect[d]	Ref.
Artemia	l + a	NAL	50	37	Growth	0	[12]
Artemia	l	NAL	100	7	Growth	–	[12]
Artemia	a	NAL	150	7	Growth	–	[12]
Artemia	l + a	TET	5	37	Growth	0	[12]
Artemia	l	TET	7.5	7	Growth	–	[12]
Artemia	a	TET	9.5	7	Growth	–	[12]
Artemia	l + a	TRI	25	37	Growth	0	[12]
Artemia	l	TRI	50	7	Growth	–	[12]
Artemia	a	TRI	75	7	Growth	–	[12]
Artemia	l + a	MET	500	37	Growth	0	[12]
Artemia	l + a	MET	1000	7	Growth	–	[12]
Calanus	a	CHL + STR	50 + 50		Respiration	0	[32]
Calanus	a	PEN	50	1	Feeding	+	[32]
Calanus	a	CHL	50	1	Feeding	–	[32]
Calanus	a	CHL + STR	50 + 50	1	Feeding	–	[32]
Calanus	a	STR	50	1	Feeding	0	[32]
Cancer	l	STR + PEN	100 + 100	40	Survival	+	[15]
Cancer	l	STR + PEN	50 + 50	40	Survival	+	[15]
Cancer	l	STR + PEN	10 + 10	40	Survival	+	[15]
Cancer	l	STR + PEN	100 + 100	100	Survival	+	[16]
Cancer	l	CHL	1	38	Survival	+	[16]
Cancer	l	CHL	5	38	Survival	+	[16]
Cancer	l	CHL	10	38	Survival	+	[16]
Cancer	l	STR + PEN	100 + 100	38	Survival	+	[16]
Cancer	l	CHL + KAN	1 + 100	30	Survival	–	[16]
Cancer	l	KAN	100	30	Survival	–	[16]
Cancer	l	CHL + NEO	1 + 50	27	Survival	–	[16]
Cancer	l	NEO	50	27	Survival	–	[16]
Cancer	l	PEN + STR	8 + 130		Survival	+	[39]
Daphnia	a	DISTR + CHL	25 + 25	1	Respiration	0	[59]
Daphnia	a	DISTR + TET	25 + 25	1	Respiration	0	[59]
Daphnia	a	DISTR + TET	50 + 50	1	Respiration	–	[59]
Eurytemora	a	CHL	20		Respiration	0	[22]
Palaemon	l	PENG + STR	30*+ 50	30–36	Survival	0	[57]
Pleuroncodes	l	PPEN + PEN	75 + 25	74	Growth	–	[5]
Temora	a	CHL	50		Growth	–	[22]

[a] Abbreviations: a = adult; l = larval

[b] Abbreviations: BAC = bacitracin; CAN = candicidin; CHL = chloramphenicol; DISTR = dihydrostreptomycin; FRA = framycetin; KAN = kanamycin; MET = methenamine-mandalate; NAL = nalidixin; NAR = naramycin; NEO = neomycin; NYS = nystatin; PEN = penicillin; PENG = penicillin G; POL = polymixin; PPEN = procaine penicillin; STR = streptomycin; TET = tetracyclin; TRI = trichomycin

[c] Days of treatment

[d] The effect is related to the parameter against the untreated control (+ positive, – negative, 0 no effect)

* Calculated from international units

3 Other Chemotherapeutics and Bactericidal Agents

Before the discovery of antibiotics chemotherapeutics were of considerable importance. However, their use in respirometry is not recommended due to their high toxicity.

Chlorine is unsuitable in respirometry and its toxicity is dependent on temperature and species [34, 44]. Significant reductions in standard respiration rates were measured in larval lobsters [7]. Increasing concentrations of residual chlorine inhibit larval development and cause morphologic deformations in some fish [36]. These effects have to be borne in mind when using chlorinated tap water, in which chlorine is more persistent than in seawater [14].

Ozone is a potent germicide [20, 37, 55]. Its efficiency is affected by temperature and pH, and it is unstable in water where the contact time is more critical than the quantity of ozone used [48]. Its toxicity is still under debate [13, 50, 56]; but due to the liberation of oxygen it interferes with the polarographic measurement.

UV-irradiation is an effective disinfectant [6], but since its penetration is limited, bacteria which adhere to the inner surfaces of the respirometer are barely affected.

4 Bacterial Growth and Respiration

Inoculation of the respirometer with bacteria occurs via the medium and the experimental animals. Bacteria are seldom freely suspended in natural waters but are generally attached to solid suspended particles [11, 35, 45]. They can be eliminated by filtering the test water (0.45 μm). Bacteria growing on the body surfaces of the test animals, however, cannot be eliminated. Rinsing the animals in a bactericide solution is not advisable, because handling and the toxicity of the solution are likely to induce stress in the animals. Bacterial respiratory inhibition by antibiotics is dependent on the growth phase and the position within a phase of growth. Therefore uniform suppression of bacterial respiration cannot be assumed [58]. If the bacteria are inhibited, fungal growth increases [12, 38]. On the other hand, antibiotics may interfere with the metabolism of the experimental animal. In some cases the total metabolism of the animal in the experiment may be considerably increased, as in *Lumbriculus variegatus* and *Salvelinus fontinalis* ([19]; Chap. II.3).

The estimation of microbial respiration in a control chamber without experimental animals is not to be recommended, because the conditions for the growth of bacteria are not identical in test and control (bacteria inoculation by experimental animals, availability of soluble excreta). The subtraction of the mean background O_2-consumption rate, i.e. (initial + final background): 2, from the oxygen uptake rate is unsuitable if the respirometer has been previously sterilized or cleaned, since this does not take into consideration the form of the bacterial growth curve during the experiment (Figs. 1 and 2).

5 Experimental Observations

A possible solution is suggested by considering the growth curve of bacteria. After an initial log-phase and following the exponential log-phase bacterial growth in a chemostats culture remains constant (stationary phase). This microbiological principle proved to be valid for bacterial growth in a respirometer.

Oxygen consumption measurements were made with an automated polarographic respirometer (Chap. II.1). This is an intermittently closed system in which any of the four chambers can be automatically closed in sequence, while flow is maintained through the other three chambers. The oxygen uptake by eight prawns (*Palaemonetes antennarius*) was recorded as the decrease in dissolved oxygen during "closed" periods of 12 min. The interval between two closed periods of the same chamber was 1 h. The prawns were repeatedly put into the respirometer for 1 h and subsequently removed for 1 h to permit measurement of the bacterial respiration in the respirometer. In this way the bacterial contamination by the experimental animals and the presence of the excreta of the prawns were guaranteed. Animals were put into the compartments of

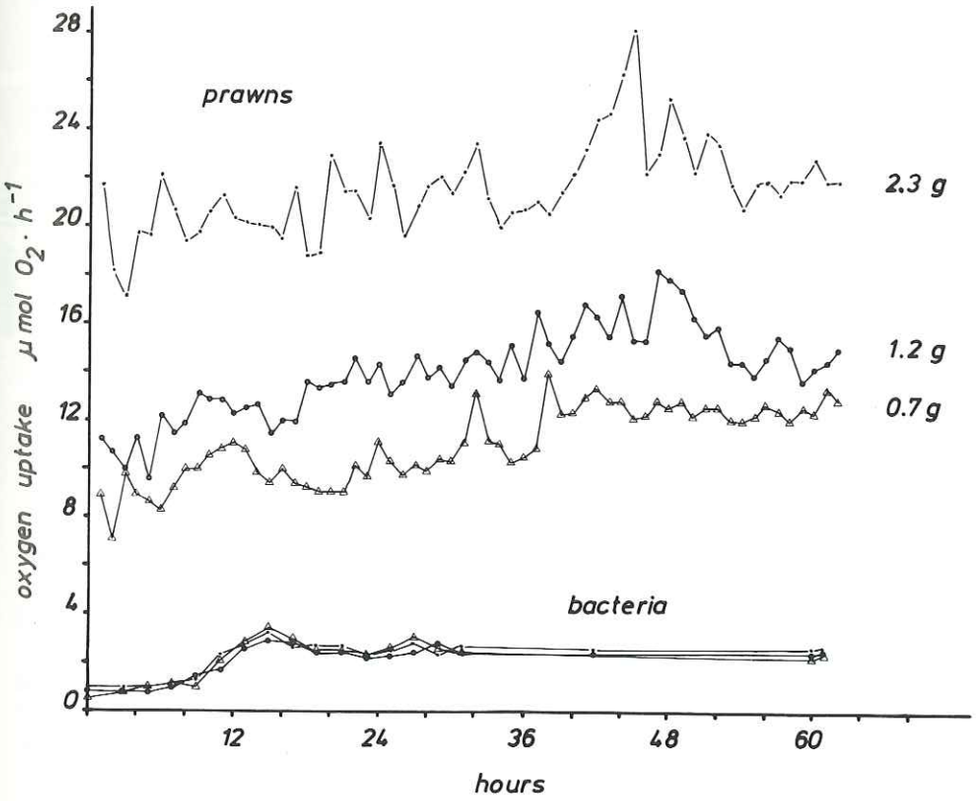

Fig. 1. Oxygen uptake of prawns uncorrected for bacterial metabolic rate and the respective simultaneous oxygen consumption rate of the bacteria in the system. The total biomass (wet weight) is given for each size group

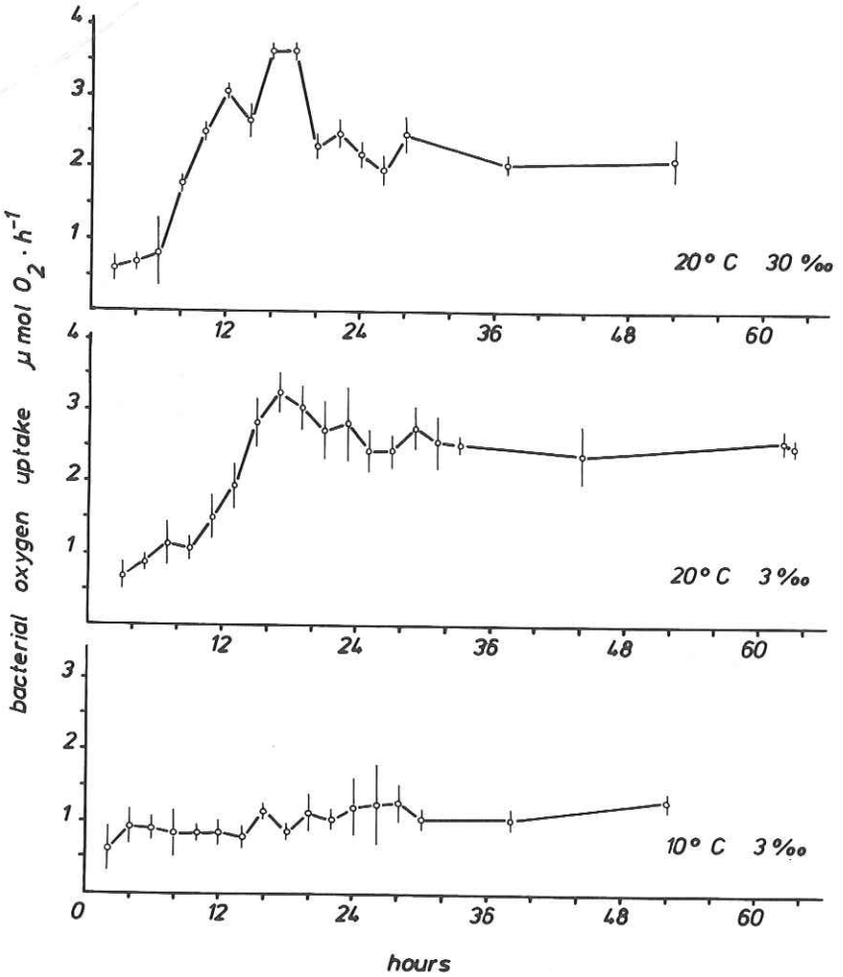

Fig. 2. The bacterial growth curve in the respirometer discontinuously inoculated with experimental animals during different conditions of salinity and temperature. Means ± S.D. are given

cages made of stainless steel netting to permit their rapid removal from the respirometer.

The bacterial growth in the respirometer increases exponentially after a lag-phase of 5 to 6 h, overshoots, and stabilizes after 12 to 20 h irrespective of animal size (Fig. 1). Hence, true animal respiration rates are obtained by determining the final bacterial background rate and substracting it from the total oxygen uptake in the experiment discharging the initial period. To estimate the initial rates, the changing bacterial respiration rate should be subtracted from the total oxygen consumption rate.

The curve of bacterial growth in the respirometer should be determined under experimental conditions. Salinity in contrast to temperature affects bacterial growth only slightly (Fig. 2). The microbial growth curve must also be determined for each

system and respirometer because the relative surface area and the materials of the respirometer exert a considerable influence [31, 33, 43]. The results also confirm that over the first 2–5 h no significant bacterial growth is observable [18] although in long-term measurements bacterial respiration can attain high levels. The bacterial oxygen consumption may be as high as 30% of that of the specimens under observation (Fig. 1). The population densities of bacteria occupying the surfaces are relatively constant and independent of the quantity of the available energy source [40, 48].

The bacterial respiratory effect is decreased by reducing the surfaces in the respirometer, avoiding plastic materials in the construction, maintaining a flow-through and increasing the biomass in the animal chamber.

6 Bacterial Interference with Stability of POS

A new membrane mounted on the POS constitutes a surface which may be gradually overgrown by bacteria. There was a correlation between deviation of the POS signal from the original calibration point and bacterial oxygen uptake (Fig. 3). This may be

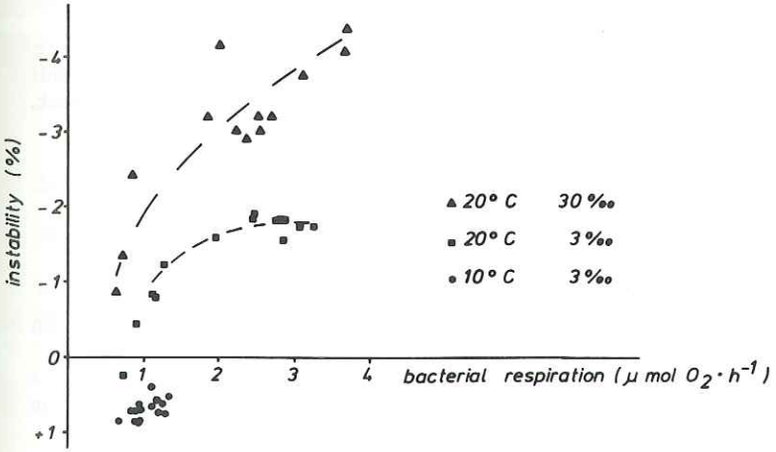

Fig. 3. Deviation of the POS stability from the beginning of the experiments as a function of bacterial respiration. Without intermittent calibrations the expected concentration of oxygen, c'_t, at time t would be

$$c'_t = I_t \times \frac{c_o}{I_o},$$

where c_o and I_o are the oxygen concentration and signal of the POS at the calibration point. The relative instability of the POS is then calculated as

$$\frac{c'_t - c_t}{c_t} \times 100.$$

The *broken lines* were fitted by eye to indicate the trends considered significant

due (1) to O_2-depletion of the membrane boundary layer by bacterial respiration, and (2) to increased thickness of the diffusion layer (membrane + bacteria) which reduces the membrane permeability. This source of error should be considered in long term in situ measurements (Part III) where uncontrolled bacterial Aufwuchs on the membrane will occur.

7 Conclusions

The bacterial problem in the respirometry of aquatic animals arises from excessive bacterial growth on the inner walls of the respirometer, especially in long-term measurements. The use of antibiotics may be considered in combatting this problem, but is limited by their physicochemical properties and because they are often more toxic to the animals than to the bacteria. Chemotherapeutics are also unsuitable in respirometry because of their high toxicity.

The time course of bacterial growth shows that the bacterial oxygen consumption rises to constant levels within 12 to 20 h and has to be subtracted from the total oxygen consumption rate. By this method no chemicals need be used to inhibit bacterial growth, thus avoiding any possible influence on the experimental animals.

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