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THE ROLE OF SULFATE EXCLUSION IN BUOYANCY MAINTENANCE BY SIPHONOPHORES AND OTHER OCEANIC GELATINOUS ZOOPLANKTON

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Abstract—1. In order to determine if a sulfate exclusion buoyancy system is operating in siphonophores, representative species were collected and analyzed for their sulfate content so that a calculated lift could be determined.

2. All the gelatinous zooplankton examined had sulfate concentrations that were significantly ($P < 0.005$) lower than local seawater samples.

3. Protein concentrations were determined in a few species and compared with the calculated lifts in order to quantitate the importance of this kind of buoyancy compensation.

4. Buoyancy maintenance mediated by the partial exclusion of sulfate appears to be important in offsetting the protein-induced negative buoyancy of gelatinous zooplankton.

5. The ecological importance of this type of buoyancy system is discussed in light of the behavior patterns of these animals.

INTRODUCTION

The siphonophores comprise a fascinating group of planktonic colonial Cnidaria whose ecology and physiological processes, until recently, have been poorly understood. Many are too delicate to survive collection in nets and trawls, and can best be studied by observing and hand-collecting them using SCUBA techniques. Their potential impact as predators (Biggs, 1976; 1977a) and capabilities for regenerating nitrogenous nutrients (Biggs, 1977b) suggest that these gelatinous zooplankton may be quantitatively important in areas or seasons in which they are locally abundant (Rogers *et al.*, 1978). Observations of their fishing behavior suggest that siphonophores are maximally effective at catching prey when they are neutrally buoyant (Mackie & Boag, 1963; Biggs, 1977a).

The marked buoyant properties of the gelatinous matrix forming the bulk of siphonophores and other gelatinous zooplankton has been noted by several authors (Jacobs, 1937; Marshall, 1954; Denton & Shaw, 1961; Denton, 1963; Mackay, 1969). Jacobs (1937) suggests that the gelatinous swimming bells and bracts of siphonophores are responsible for offsetting the weight of their heavy feeding and reproductive parts. In addition, certain siphonophores are apparently able to regulate their buoyancy by becoming lighter or heavier than a surrounding seawater medium in the course of approximately 1 hr (Jacobs, 1937).

Potential strategies which may be utilized by a marine organism in order to counteract sinking include: active swimming movements, use of a gas-filled swimbladder or float, a high surface-area to volume ratio, accumulation of low density organic compounds (e.g. fats, oils) and the selective exclusion of heavy ions. A wide diversity of marine biota employ the latter mechanism to regulate their buoyancy. Upon lowering the density of their internal fluids by isosmotically replacing heavier ions with lighter species, these marine organisms can obtain a positive lift. For example, the marine dinoflagellate *Pyrocystis noctiluca* (Kahn & Swift, 1978), larval tunicates (Lambert & Lambert, 1978) and cranchiid squid (Denton *et al.*, 1969) all accumulate ammonium ions while eliminating heavier cations (e.g. Na^+, Ca^{2+}, Mg^{2+}).

Sulfate exclusion coupled with an isosmotic replacement with the chloride ion has been documented in most of the gelatinous zooplankton examined to date (Robertson, 1949; Denton & Shaw, 1961). Although a variety of gelatinous taxa have been analyzed for their sulfate content and observed lift (Denton & Shaw, 1961), no comparable data are available for the Siphonophora. We suspected that this type of buoyancy system might provide significant lift for siphonophores. The small volume of the gas-filled float present in many of the physonect siphonophores (e.g. *Agalma okeni*, *Forskalia edwardsi*) suggests that an alternate buoyancy mechanism may be quantitatively more important in these animals (Jacobs, 1937; Biggs, 1977a). Moreover, the ability to regulate their buoyancy should be important in maximizing their prey-capturing effectiveness, since most siphonophores fish for prey with their network of fishing tentacles extended in cylindrical or spherical configurations like three dimensional spider webs (Biggs, 1977a). Rising or sinking changes the configuration of the extended web of tentacles and decreases the volume of water it controls (Mackie & Boag, 1963). Any active swimming movements produced by these animals while fishing might alert potential prey from contact with their fishing tentacles. In addition to facilitating feeding, a sulfate exclusion buoyancy system may be used during diel vertical migration to reduce the amount of energy necessary for upward swimming movements.

In order to determine if a sulfate exclusion buoyancy system is operating in siphonophores, representative species were collected and analyzed for their sulfate content so that a calculated lift could be determined. Comparative data were also collected for other abundant taxa of gelatinous zooplankton. Protein concentrations were determined in a few species and compared with the calculated lifts in order to quantitate the importance of this kind of buoyancy compensation.

MATERIALS AND METHODS

As mentioned above, the fragility of many siphonophores prohibits the use of nets and trawls for their collection. Accordingly, most of the siphonophores and other gelatinous zooplankton used in this study were carefully enclosed in hand-held jars by SCUBA divers as detailed by Hamner *et al.* (1975). Collections were made at a series of SCUBA transect stations occupied during Atlantis-II 101 cruise (June–July 1978; see Fig. 1). Less fragile siphonophore species (*Vogtia glabra*, *V. spinosa* and *Hippopodius hippopus*) were collected intact with a midwater trawl. Once collected and identified, the animals were placed in large flow-through aquaria which maintained the samples at ambient seasurface temperature (±2°C). Within 6 hr after collection, individual animals were carefully removed from their jars using a perforated tablespoon. After a quick rinse with distilled water the tablespoon with animal was blotted on absorbant paper to remove excess water. Single specimens or pooled individuals of a given species were homogenized using an electric drill equipped with a teflon tissue grinder. The homogenate was then centrifuged for 1 hr at 4000 rpm using a Sorvall SS4 centrifuge. Aliquots of the clear supernatant were removed by pipette and diluted for sulfate determination.

Local seawater samples and aliquots taken from the supernatant dilutions were analyzed for sulfate using a turbidimetric method adapted from the technique outlined in *Standard Methods for the Examination of Water and Wastewater* (1975). Linear results were obtained for calibration curves ranging from 10–35 mg SO_4^{2-}/l with an experimental error of ±5% (i.e. 20 ± 1 mg/l). Theoretical lifts were calculated by assuming that the sulfate excluded, relative to seawater, was replaced isosmotically with chloride (Robertson, 1949; Denton & Shaw, 1961). The theoretical lift calculations were made using the freezing-point depression (Δ) data given for sodium chloride and sodium sulfate in the *CRC Handbook of Chemistry and Physics* (51st edition). Ionic partial molal volumes were not taken into account in these calculations since the partial molal volume of chloride is similar to that of the sulfate ion (Millero, 1969).

In order to quantitate the importance of sulfate exclusion in buoyancy maintenance, the lift obtained by the partial elimination of sulfate was compared to the protein content of two species: *Beroe cucumis* (a ctenophore) and *Pelagia noctiluca* (a scyphozoan jellyfish). Protein constitutes the major organic fraction in marine zooplankton (Reeve *et al.*, 1970; Ikeda, 1972; Mayzaud & Martin, 1975). Since its density is greater than seawater, protein has the potential of producing a negative buoyancy in these animals. A Percent Compensation Index (PCI) was calculated for *B. cucumis* and *P. noctiluca* as follows:

$$\text{PCI} = \frac{\text{(lift obtained by the partial exclusion of sulfate)}}{|\text{(negative buoyancy produced by protein)}|} \times 100$$

where both the calculated lift and negative buoyancy created by the protein are given in units of mg/ml. The weight of the animal protein in seawater was calculated as detailed by Denton & Marshall (1958) assuming the specific gravity of protein and the density of seawater to be 1.3 (MacDonald, 1975) and 1.025 g/cm^3 respectively. Protein determinations were carried out using the Lowry *et al.* (1951) method with freeze-dried bovine serum albumin (BSA) as the reference standard. The seawater densities were measured with a 5 ml pycnometer at 25°C.

RESULTS

Sulfate values for seawater samples and gelatinous zooplankton examined are given as the mean ± the standard error ($\bar{x} \pm s_{\bar{x}}$). The seawater samples analyzed had an average sulfate concentration of 3.2 mg SO_4^{2-}/ml whereas the sulfate levels in the animals were significantly lower ($P < 0.005$) with a range of 0.8–2.2 mg SO_4^{2-}/ml of supernatent (see Table 1). One

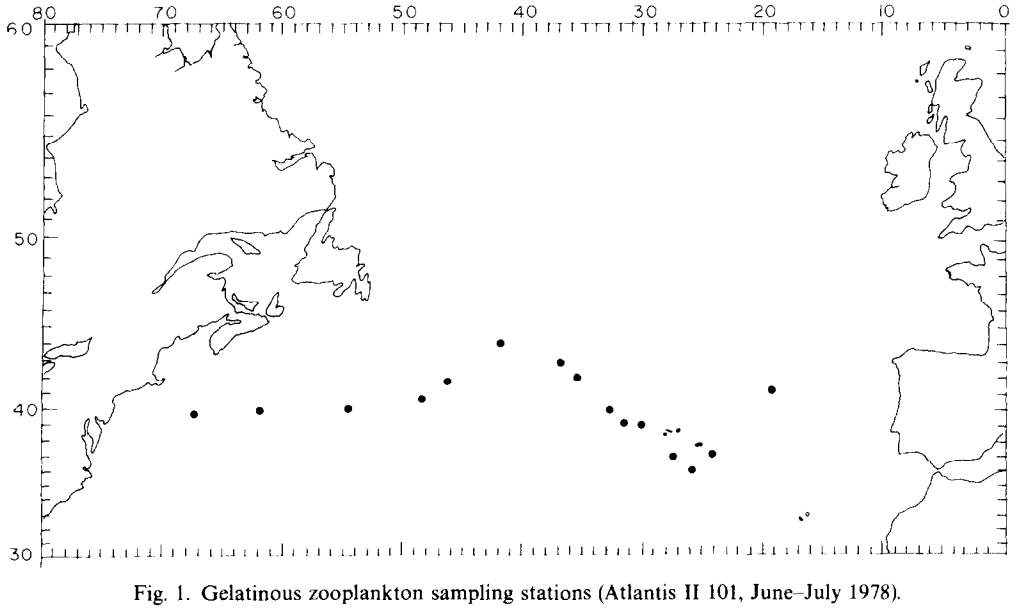

Fig. 1. Gelatinous zooplankton sampling stations (Atlantis II 101, June–July 1978).

Table 1. Sulfate concentrations and calculated lifts of the gelatinous zooplankton examined

Sample	n	mg SO_4^{2-} ml^{-1} ($\bar{x} \pm s_{\bar{x}}$)	Calculated lift (mg ml^{-1}) ($\bar{x} \pm s_{\bar{x}}$)	% SO_4^{2-} excluded ($\bar{x} \pm s_{\bar{x}}$)
seawater	7	3.2 ± 0.1	-	
Phylum Cnidaria				
Order Siphonophora				
sub-order Physonectae				
Agalma okeni	2	1.8 ± 0.1	1.0 ± 0.1	44 ± 3
Forskalia edwardsi	1	1.8 ± 0.1	1.0 ± 0.1	44 ± 3
sub-order Calycophorae				
Diphyes dispar	5	0.8 ± 0.2	1.6 ± 0.1	75 ± 6
Hippopodius hippopus	4	1.1 ± 0.1	1.4 ± 0.1	66 ± 3
Rosacea cymbiformis	3	1.2 ± 0.2	1.4 ± 0.1	63 ± 5
Stephanophyes superba	4	1.8 ± 0.2	1.0 ± 0.2	45 ± 8
Vogtia glabra	3	1.9 ± 0.2	0.9 ± 0.2	41 ± 8
Vogtia spinosa	4	1.8 ± 0.1	1.0 ± 0.1	43 ± 3
Order Semaeostomeae				
Aurelia aurita	2	1.8 ± 0.2	1.0 ± 0.2	44 ± 6
Pelagia noctiluca	4	2.0 ± 0.1	0.8 ± 0.1	38 ± 4
Phylum Ctenophora				
Order Beroida				
Beroe cucumis	6	1.4 ± 0.1	1.2 ± 0.1	55 ± 3
Order Cestida				
Cestum veneris	2	2.2 ± 0.2	0.7 ± 0.1	33 ± 5
Phylum Chordata				
Order Salpida				
Cyclosalpa pinnata	2	1.8 ± 0.1	1.0 ± 0.1	46 ± 2

of the calycophore siphonophores, *Diphyes dispar*, excluded 75% of its sulfate relative to seawater. The calculated lifts ranged from 0.7 to 1.6 mg/ml.

A comparison of the protein and sulfate concentrations present in *Beroe cucumis* and *Pelagia noctiluca* showed that *P. noctiluca* had nearly three times the protein concentration found in *B. cucumis* (see Table 2). However, *B. cucumis* excluded one and a half times more sulfate than did *P. noctiluca* to obtain a lift of 1.2 mg/ml. By excluding 55% of its sulfate relative to seawater, *B. cucumis* was capable of offsetting all of its protein mass present (PCI = 279%). *Pelagia noctiluca*, on the other hand, was only capable of offsetting 66% of its protein mass by the exclusion of 38% of its sulfate content (see Table 2).

DISCUSSION

The data presented in Table 1 suggest that a sulfate exclusion buoyancy mechanism is operating in the siphonophores and other gelatinous zooplankton examined. The relatively low standard error ($s_{\bar{x}}$) associated with these numbers suggest that this buoyancy mechanism operates in a fairly uniform fashion in members of a given species. The percent sulfate exclusion value given for *Beroe cucumis* is not significantly different from that reported by Denton & Shaw (1961) for *B. forskalia* and *B. ovata*, though their value for *Pelagia noctiluca* is somewhat higher than the number given here. Robertson (1949) found that *Aurelia aurita* excluded 53% of its sulfate content relative to seawater which is comparable to the value of 44% ± 6% determined in our study.

It is interesting to note that most of the calycophore siphonophores (gas-filled float absent) examined tend to exclude more sulfate than the physonect siphonophores (gas-filled float present). These data indirectly suggest that the gas-filled float may play a role in buoyancy maintenance. The importance of the gas-filled float in siphonophores is discussed in more detail by Jacobs (1937) and others (Marshall, 1954; Wittenberg, 1960; Hahn & Copeland, 1966; Copeland, 1968; Pickwell, 1970; Biggs, 1977a).

Table 2. Significance of sulfate exclusion in buoyancy maintenance in *Beroe cucumis* and *Pelagia noctiluca*

Sample	n	mg Protein ml^{-1} ($\bar{x} \pm s_{\bar{x}}$)	Protein lift (mg ml^{-1}) ($\bar{x} \pm s_{\bar{x}}$)	Sulfate exclusion lift (mg ml^{-1}) ($\bar{x} \pm s_{\bar{x}}$)	PCI ($\bar{x} \pm s_{\bar{x}}$)
Beroe Cucumis	5	2.1 ± 0.2	-0.4 ± 0.1	1.2 ± 0.1	279 ± 35
Pelagia noctiluca	2	5.9 ± 1.0	-1.2 ± 0.2	0.8 ± 0.2	66 ± 24

The ctenophore, *Cestum veneris*, excludes the least amount of sulfate in comparison with the other zooplankton examined. When considering the observation that *C. veneris* is not an active swimmer (Harbison *et al.*, 1978), one wonders how this ctenophore is able to maintain itself in the water column without sinking. Perhaps the frictional drag produced by its relatively high surface-area to volume ratio drastically reduces its sinking rate.

An interesting comparison based on the degree of sulfate exclusion, protein concentration and behavior can be made between *Beroe cucumis* and *Pelagia noctiluca*. *Beroe cucumis*, which is a relatively poor swimmer, excludes enough sulfate to fully compensate for the negative buoyancy produced by its protein. *Pelagia noctiluca*, on the other hand, is a very active swimmer and in this case sulfate exclusion compensates for only 66% of its protein mass. These data suggest that *B. cucumis* relies on the partial exclusion of sulfate to remain buoyant while *P. noctiluca* uses both active swimming movements and sulfate exclusion to maintain itself in the water column. The importance of active swimming movements used by *P. noctiluca* to avoid sinking is reflected by its high protein content.

Buoyancy maintenance mediated by a sulfate exclusion mechanism appears to provide enough lift to offset most or all of the protein-induced negative buoyancy in gelatinous zooplankton. In addition, this mechanism could be used to adjust the density of these animals if their sulfate content was controlled by an active transport system as suggested by the work of Mackay (1969). Ammonium accumulation coupled with the exclusion of sodium does not appear to be important in providing lift because of the low ammonium ion concentration present in the gelatinous zooplankton we examined (≤ 0.5 mM NH_4^+). Furthermore, a significant lift is probably not obtained by the accumulation of lipids because of the low lipid content of gelatinous zooplankton (Sipos *et al.*, 1968; Hooper *et al.*, 1972). Additional lift, however, might be obtained by the selective exclusion of other heavy ions which have a large negative partial molal volume (e.g. Ca^{2+}, Mg^{2+}). Turbulence may also be important in keeping these animals suspended in the water column.

The exact mechanism of sulfate exclusion in gelatinous zooplankton remains to be determined. It would be interesting to see if this mechanism is similar to the model proposed by Cabantchik *et al.* (1978) for the anion transport system of the red blood cell. Perhaps sulfate exclusion in these animals is facilitated by the production and secretion of sulfate-rich mucopolysaccharides. Many gelatinous zooplankton (e.g. ctenophores, scyphozoan jellyfish), in addition to other marine invertebrates, produce large amounts of mucus when feeding or stressed (personal observation; Hunt, 1970; Harbison *et al.*, 1978). By understanding buoyancy mechanisms in the gelatinous zooplankton we may get a better picture of the feeding and migrating patterns of these animals.

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REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION. (1975) *Standard Methods for the Examination of Water and Wastewater*, 14th edn, pp. 496–498. American Public Health Association, Washington, D.C.

BIGGS D. C. (1976) Nutritional ecology of *Agalma okeni*. In *Coelenterate Ecology and Behavior*, (Edited by MACKIE G. O.) pp. 201–210. Plenum, New York.

BIGGS D. C. (1977a) Field studies of fishing, feeding and digestion in siphonophores. *Mar. behav. Physiol.* **4**, 261–274.

BIGGS D. C. (1977b) Respiration and ammonium excretion by open ocean gelatinous zooplankton. *Limnol. Oceanog.* **22**, 108–117.

CABANTCHIK Z. I., KNAUF P. & ROTHSTEIN A. (1978) The anion transport system of the red blood cell. The role of membrane protein evaluated by the use of 'probes'. *Biochem. biophys. Acta* **515**, 239–302.

COPELAND O. E. (1968) Fine structures of the carbon monoxide secreting tissue in the float of Portuguese man-of-war *Physalia physalia* L. *Biol. Bull.* **135**, 486–500.

DENTON E. J. (1963) Buoyancy mechanisms of sea creatures. *Endeavour.* **22**, 3–8.

DENTON E. J., GILPIN-BROWN J. B. & SHAW T. I. (1969) A buoyancy mechanism found in cranchiid squid. *Proc. Roy. Soc. B.* **174**, 271–279.

DENTON E. J. & MARSHALL N. B. (1958) The buoyancy of bathypelagic fish without a gas-filled swimbladder. *J. mar. Biol. Ass. U.K.* **37**, 753–767.

DENTON E. J. & SHAW T. I. (1961) The buoyancy of gelatinous marine animals. *J. Physiol. Lond.* **161**, 14–15.

HAHN W. E. & COPELAND O. E. (1966) Carbon monoxide concentration and the effect of aminopterin on its production in the gas bladder of *Physalia physalia*. *Comp. Biochem. Physiol.* **18**, 201–207.

HAMNER W. M., MADIN L. P., ALLDREDGE A. L., GILMER R. W. & HAMNER P. P. (1975) Underwater observations of gelatinous zooplankton: Sampling problems, feeding biology and behavior. *Limnol. Oceanog.* **20**, 907–917.

HARBISON G. R., MADIN L. P. & SWANBERG N. R. (1978) On the natural history and distribution of oceanic ctenophores. *Deep-Sea Res.* **25**, 233–256.

HOOPER S. N. & ACKMAN R. G. (1972) Presence of trans-6-hexadecenoic acid in the white jellyfish *Aurelia aurita*. *Lipids* **7**, 624–626.

HUNT S. (1970) *Polysaccharide-protein Complexes in Invertebrates*, p. 55. Academic Press, London.

IKEDA T. (1972) Chemical composition and nutrition of zooplankton in the Bering Sea. In *Biological Oceanography of the Northern North Pacific Ocean*, (Edited by A. Y. TAKENOUTI *et al.*) pp. 433–442. Idemitsu.

JACOBS E. (1937) Beobachtungen uber das schweben der siphonophoren. *Z. vergl. Physiol.* **24**, 583–601.

KAHN N. & SWIFT E. (1978) Positive buoyancy through ionic control in the non-motile marine dinoflagellate *Pyrocystis noctiluca*. *Limnol. Oceanog.* **23**, 649–658.

LAMBERT C. C. & LAMBERT G. L. (1978) Tunicate eggs utilize ammonium ions for flotation. *Science* **200**, 64–65.

LOWRY O. H., ROSEBROUGH N. J., FARR A. L. & RANDALL R. J. (1951) Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193,** 265–275.

MACDONALD A. G. (1975) *Physiological Aspects of Deep Sea Biology*, p. 239. Cambridge University Press, Cambridge.

MACKAY W. C. (1969) Sulphate regulation in jellyfish. *Comp. Biochem. Physiol.* **30,** 481–488.

MACKIE G. O. & BOAG D. M. (1963) Fishing, feeding, and digestion in siphonophores. *Pubbl. Staz. Zool. Napoli.* **33,** 178–196.

MARSHALL N. B. (1954) *Aspects of Deep Sea Biology*, pp. 185–214. Hutchinson, London.

MAYZAUD P. & MARTIN J.-L. (1975) Some aspects of the biochemical and mineral composition of marine plankton. *J. exp. mar. Biol. Ecol.* **17,** 297–310.

MILLERO F. J. (1969) The partial molal volume of ions in seawater. *Limnol. Oceanog.* **14,** 376–385.

PICKWELL G. V. (1970) The physiology of carbon monoxide production by deep-sea coelenterates: Causes and consequences. *Ann. N.Y. Acad. Sci.* **174,** 102–115.

REEVE M. R., RAYMONT J. E. & RAYMONT J. K. (1970) Seasonal biochemical compositional and energy sources of *Sagitta hispida*. *Mar. Biol.* **6,** 357–364.

ROBERTSON J. D. (1949) Ionic regulation in some marine invertebrates. *J. exp. Biol.* **26,** 182–200.

ROGERS C. A., BIGGS D. C. & COOPER R. A. (1978) Aggregation of the siphonophore *Nanomia cara* in the Gulf of Maine: Observations from a submersible. *Fish. Bull.* **76,** 281–284.

SIPOS J. C. & ACKMAN R. G. (1968) Jellyfish (*Cyanea capillata*) lipids: Fatty acid composition. *J. fish. Res. Bd. Canada.* **25,** 1561–1569.

WEAST R. C. (Editor) (1970–71) *Handbook of Chemistry and Physics*, 51st edition, pp. D181–D226. The Chemical Rubber Company, Cleveland, Ohio.

WITTENBERG J. B. (1960) The source of carbon monoxide in the float of the Portuguese man-of-war, *Physalia physalia* L. *J. exp. biol.* **37,** 698–705.