Ammonia and Inorganic Phosphorus Excretion by the Planktonic Chaetognath, *Sagitta hispida* Conant[1]

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Introduction

The chaetognaths are a common group of carnivores in many marine zooplankton populations. MENZEL and RYTHER (1961) found that on an average yearly basis chaetognaths composed the second largest fraction (15·1 %), next to copepods (44·2%), in terms of the percentage dry weight of the total zooplankton population in the upper 500 m at a station occupied biweekly in the Sargasso Sea near Bermuda. Despite their abundance, our knowledge of the physiology of the chaetognaths is extremely limited (HYMAN, 1959). This is due primarily to the inability of most species to survive in the laboratory. However, observations on the neritic form, *Sagitta hispida* Conant, have indicated that this species can adapt to laboratory conditions (BEERS, unpublished). *S. hispida* is a relatively small (average length 10–12 mm), rigid chaetognath. It abounds within the reef area of Bermuda and can be found as a conspicuous member of the zooplankton population throughout most of the year.

In the present study, the rates of excretion of ammonia and inorganic phosphorus by *S. hispida* have been investigated. In addition, the total Kjeldahl-nitrogen content of the animals was determined and has been related to the amount of nitrogen liberated by ammonia excretion.

Materials and Methods

Sea water

Sea water used for the maintenance of animals and for the actual experimental work was from the surface of the open ocean (salinity approximately 36·5‰) around Bermuda. This was filtered through Whatman glass filters (GF/C) and stored in polyethylene carboys, generally for less than one week. Before

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use, 50 mg dihydrostreptomycin sulphate/l (Glaxo) was added to combat bacterial growth. This water will, henceforth, be referred to as the "experimental sea water." MARSHALL and ORR (1961) found that the addition of streptomycin at this concentration had no apparent harmful effect on the feeding of *Calanus finmarchicus*. Also, MARSHALL and ORR (1958) reported no significant difference in oxygen consumption rates of female *C. finmarchicus* in water with 50 mg/l of both streptomycin and chloromycetin and water without added antibiotics.

Animals

*Sagitta hispida** were obtained by net tows made in St. George's Harbor, Bermuda, usually soon after sunset. A 0·75 m No. 2 mesh net was towed at low speed for 10–15 minutes just below the surface. The concentrated plankton were immediately diluted with a large volume of surface water, and within three hours of the time of collection the chaetognaths were separated out and placed in the experimental sea water. Unless otherwise noted, they were maintained in this water for approximately 24 hours under darkened conditions at $20 \pm 1°C$ before being used in the experimental work. Generally, 70–80% of the animals would be in good condition at the end of this period. *S. hispida* could be maintained in the laboratory in an apparently healthy state for at least two weeks, and would feed readily on brine shrimp (*Artemia* sp.) larvae.

During the 24-hour period of adaptation the animals were not fed. Only those with apparently empty digestive tracts were used in the excretion studies. PARRY (1944), studying the chaetognath, *Spadella cephaloptera*, found that the time between ingestion and defecation, when fed copepods, was 3–4 hours. DAVID (1955) reported that the remains of a euphausid fed to a *Sagitta gazellae* reached the anus in approximately one hour. If food is passed through the digestive tract of *S. hispida* at a comparable rate, no food materials should have remained in the gut at the end of the period of adaptation. Therefore, the rates of excretion determined for *S. hispida* should be considered as "basal" rates.

All animals used were in stages II and III of maturity, i. e. animals with clusters of sperm cells in the seminal vesicles (PIERCE, 1951).

Experimental

Rates of excretion were determined from the difference in the ammonia and phosphate levels in sea water in which the animals were maintained for a 24 hour period, and duplicate sea water samples containing no animals. All work was done in glass-stoppered pyrex bottles having a volume of approximately 158 ml. The bottles were rinsed with one or two aliquots of the experimental sea water and then filled to volume. The animals were "rinsed" by passing them through several changes of this water. They were then transferred with a minimum volume of water to the filled bottles.

A minimum of three bottles containing no animals were used as the controls in each series.

The bottles were tightly stoppered and maintained for a period of 24 hours under conditions of complete darkness and at a temperature of $20 \pm 1°C$.

*) The identification of this species by Dr. E. LOWE PIERCE, University of Florida, Gainesville, is gratefully acknowledged.

Following this, the animals were rapidly removed and the water frozen in polyethylene bottles for chemical analyses at a later date, generally within 3–4 days. The animals were counted, the length of the individuals measured, and the dry weight of each group determined collectively. The animals were dried to a constant weight at a temperature of approximately 60°C.

Chemical analyses

Quantitative determination of the ammonia-N content of the water samples was by the colorimetric method of KRUSE and MELLON (1952). An additional optical density reading at 600 mμ was used as a sea water "turbidity" correction (MENZEL, unpublished). The standard deviation of twelve replicates of a standard $(NH_4)_2SO_4$ solution containing 1·00 μgA NH_3-N/l was ± 0·13. At 4·00 μgA NH_3-N/l, the standard deviation was ± 0·25. Inorganic phosphorus was measured by the method of MURPHY and RILEY (1958). Standard deviation at 0·25 μgA PO_4-P/l was ± 0·02 and ± 0·02 at 0·50 μgA PO_4-P/l (six replicates). Total Kjeldahl-nitrogen content of dried specimens of *S. hispida* was determined by a micro-procedure based on the method of PERRIN (1953). The deviation for seven samples of "Casilan" (calcium caseinate, Glaxo) and six casein (Nutritional Biochemicals Corp.) samples of 8–16 mg each was less than ± 2%.

The level of nitrate + nitrite-N was measured by the method of MULLIN and RILEY (1955) in experiments 1–10 since VACCARO (1961) gave evidence that ammonia may be oxidized to nitrite and nitrate within a 24-hour period through unknown chemical processes. The results of these will not be considered since the observed differences between bottles with animals and the controls were all small and within the error that would be expected due to the reproducibility of the method and, therefore, cannot be considered significant.

Results

Results of all determinations of the excretion of ammonia-N and phosphate-P by *Sagitta hispida* are summarized in Table 1. In finding the averages, only the results from bottles in which all animals survived the 24-hour period were used. The mean rate of ammonia excretion by animals subjected to the standard experimental conditions was 0·10 μgA NH_3-N/animal/24 hours or 0·91 μgA NH_3-N/mg dry weight/24 hours (Table 1). Inorganic phosphorus excretion by these animals was, on the average, 0·008 μgA PO_4-P/animal/24 hours and 0·077 μgA PO_4-P/mg dry weight/24 hours (Table 1).

Greater differences were observed in the rates of both ammonia and phosphorus excretion in some experiments than would be expected due to the reproducibility of the colorimetric methods employed in analysing the water. In Table 1 the standard deviation for the ammonia excretion rates determined on the basis of the animal's dry weight is shown. These variations may reflect differences in the general nutritional state of the animals, although this is merely speculation at the present time.

A higher excretion rate calculated on the basis of the dry weight of the animals was found in the bottles with four or less organisms (Experiments 10 and 11, Table 1). While not conclusive due to the small number of determinations and the large standard deviation, it is conceivable that the higher rates may be

Table 1

Ammonia-N and phosphate-P excretion by *Sagitta hispida*

Experiment and date			Number of bottles	Average number animals/bottle	µg A NH_3-N/ animal/ 24 hours	µg A NH_3-N/ mg dry weight/ 24 hours ± standard deviation	µg A PO_4-P/ animal/ 24 hours	µg A PO_4-P/ mg dry weight/ 24 hours	NH_3-N: PO_4-P excretion/ mg dry weight/ 24 hours (by atoms)
1. 27. October 1961			2(0)	7	0·08	1·12 ± ·05	0·006	–	– –
2. 4. November 1961			3(1)	7	0·11	1·43 ± ·23	0·011	0·129	11·1 : 1
3. 15. November 1961			3(0)	7	0·12	1·40 ± ·33	0·008	0·098	14·3 : 1
4. 17. November 1961			6(0)	6	0·14	0·94 ± ·31	0·010	0·069	13·6 : 1
5. 12. January 1962			2(5)	6	0·11	0·73 ± ·03	0·008	0·059	12·4 : 1
6. 16. January 1962		+	7(0)	10	0·07	0·56 ± ·07	0·006	0·051	11·0 : 1
7. 22. January 1962		+	6(0)	12	0·06	0·45 ± ·06	0·006	0·043	10·5 : 1
8. 24. February 1962	a)	*	3(1)	9	0·10	1·17 ± ·23	0·013	0·144	8·1 : 1
	b)		4(0)	9	0·12	1·39 ± ·21	0·010	0·128	10·9 : 1
9. 9. March 1962			3(3)	9	0·09	0·68 ± ·12	0·009	0·066	10·3 : 1
10. 21. March 1962	a)		3(0)	2	0·09	1·37 ± ·10	0·006	0·091	15·1 : 1
	b)		3(0)	4	0·11	1·25 ± ·43	0·012	0·139	9·0 : 1
	c)		2(1)	6	0·12	1·01 ± ·20	0·006	0·063	16·0 : 1
11. 10. April 1962	a)		3(0)	2	0·12	0·89 ± ·04	0·014	0·109	8·2 : 1
	b)		2(1)	5	0·10	0·58 ± ·26	0·010	0·053	10·9 : 1
	c)		2(1)	8	0·09	0·61 ± ·01	0·009	0·063	9·7 : 1
12. 29. May 1962			10(0)	4	0·06	0·53 ± ·20	0·005	0·040	13·3 : 1
13. 30. May 1962		0	3(2)	4	0·11	0·75 ± ·37	0·009	0·063	11·9 : 1
14. 8. June 1962	a)	+	4(1)	4	0·11	0·65 ± ·23	0·014	0·093	7·0 : 1
	b)		4(0)	4	0·06	0·44 ± ·10	0·011	0·076	5·8 : 1
	c)	*	4(0)	4	0·11	0·67 ± ·30	0·014	0·090	7·4 : 1
15. 16. June 1962	a)		4(0)	4	0·12	0·93 ± ·15	0·007	0·052	17·9 : 1
	b)	*	4(2)	4	0·13	0·98 ± ·26	0·009	0·074	13·2 : 1
16. 20. June 1962	a)	+	4(1)	4	0·05	0·30 ± ·06	0·004	0·025	12·0 : 1
	b)		5(0)	4	0·06	0·37 ± ·08	0·005	0·035	10·6 : 1

( ) – Number of additional bottles having one or more dead animals at end of 24-hour period. Not included in determination of excretory rates.
* – Water sample filtered at end of experimental period.
\+ – No period of adaptation to laboratory conditions.
0 – 48-hour period of adaptation to laboratory conditions.

normal and that the excretion in bottles with large numbers of organisms may be reduced due to a toxic effect of the level of excretory products in the water.

The effect upon the measured level of ammonia due to filtering the sea water through glass filters (Whatman GF/C) immediately after removing the animals following the experimental period was examined in three series. Although no fecal material or other particulate matter was macroscopically visible, filtering should remove any such material formed during the experimental period. No marked difference between filtered and unfiltered samples was noted (Experiments 8, 14 (phosphate) and 15 (ammonia), Table 1). However, the ammonia-N concentration in Experiment 14 and the phosphate-P in Experiment 15 were considerably higher in the filtered water. The explanation for this is unknown.

The mean ratio of NH_3-N:PO_4-P excretion (by atoms) was found to be 11·3 : 1 when calculated on the basis of excretion/mg animal dry weight in 24 hours (Table 1).

Results of three series of determinations of the Kjeldahl-nitrogen level of *S. hispida* are summarized in Table 2. Mean nitrogen content was found to be 87·3 µg/mg dry weight or 6·23 µg A nitrogen/mg dry weight. Thus, the mean excretion of 0·91 µg A ammonia-N/mg dry weight during the 24-hour experimental period represents the loss of approximately 14·5% of the nitrogen content.

Table 2

Total Kjeldahl-nitrogen content of *Sagitta hispida*

Experiment	Date	Number of samples	Average mg Kjeldahl-N/ mg dry weight	Average µg A Kjeldahl-N/ mg dry weight
1	16. March 1962	5	0·087	6·20
2	21. March 1962	6	0·089	6·32
3	11. April 1962	7	0·086	6·15

Discussion

Few studies have been made of the production of ammonia by living zooplankton. HARRIS (1959), investigating various aspects of the nitrogen cycle in Long Island Sound, found an average excretion of 2·60 µg A NH_3-N/mg dry weight/24 hours in freshly collected mixed populations whose excretion was determined over a 4-hour period.

The excretion of inorganic phosphorus has been studied in several planktonic forms. HARRIS (1959) showed a daily mean production of 0·37 µg A PO_4-P/mg dry weight by the Long Island Sound zooplankton. The freshwater crustacean, *Daphnia magna*, excretes inorganic phosphorus at the rate of 0·032 µg/mg dry weight/hour or 0·025 µg A/mg dry weight/24 hours (RIGLER, 1961). From the data of CUSHING (1954) and the calculations of RIGLER (1961), the inorganic phosphorus excretion of the marine copepod, *Calanus finmarchicus*, can be estimated to be 0·037 µg A/mg dry weight/24 hours.

The differing conditions under which the rates of ammonia and inorganic phosphorus excretion have been measured in the examples cited make a valid comparison with the present work difficult. However, on the basis of excretion per unit animal dry weight they are of the same order as determined for *Sagitta hispida*. It should be re-emphasized that the chaetognaths were given a 24-hour

period of adaptation to laboratory conditions without feeding prior to the determination of their excretory rates, and that the results are best considered as a "basal" level of production of ammonia and inorganic phosphorus.

The production of excretory products by chaetognaths and other planktonic forms is of significance in studying the cycles of biologically important elements in the sea. Data from the limited number of studies on zooplankton excretion have indicated the considerable importance of their excretory products in the regeneration of essential nutrients in marine waters (KETCHUM, 1962).

GARDINER (1937), HARRIS (1959) and POMEROY, MATHEWS and MIN (1963) have suggested that the inorganic phosphorus excreted by zooplankton may provide a significant amount of that required by the phytoplankton.

HARRIS (1959) calculated that 43–66% of the daily nitrogen requirements of the phytoplankton in regions of Long Island Sound may be provided by zooplankton ammonia production.

In general, ammonia is the major nitrogenous excretory product of marine invertebrates (PROSSER and BROWN, 1961). ZOBELL (1935) and COOPER (1937) indicated that ammonia can serve as a direct source of nitrogen for phytoplankton growth. HARRIS (1959) showed that the most rapid growth in mixed phytoplankton populations from Long Island Sound was often observed in ammonia-enriched sea water in contrast to that enriched with nitrate or nitrite. GUILLARD (1963) reported the growth of several species of bacteria-free diatoms and flagellates in sea water enriched with ammonia.

Studies by DUGDALE and DUGDALE (1962) have indicated a rapid turnover rate of ammonia in the euphotic zone of the Sargasso Sea. From data on the mean ammonia concentration at 10 m (MENZEL and SPAETH, 1962) and the mean uptake rate of ammonia by naturally occurring populations, they estimated that, in the absence of recycling mechanisms, the available ammonia would be depleted in less than four days.

No simultaneous determinations of the rate of turnover of ammonia and the regeneration through zooplankton excretion have been made. Further work on the excretion of a wider variety of zooplankton forms with accompanying data on the abundance of these organisms is needed before the importance of zooplankton excretory products in the cycle of nitrogen can be evaluated.

Summary

Ammonia-nitrogen and phosphate-phosphorus excretion was studied in the chaetognath, *Sagitta hispida* Conant. Rates of excretion were determined by the difference in ammonia and phosphate levels in sea water in which the animals were maintained for a 24-hour period and duplicate sea water samples with no animals.

The average daily rate of ammonia-N excretion by *S. hispida* was 0·91 µg A/mg dry weight. Phosphate-P excretion averaged 0·077 µg A/mg dry weight/day. The ratio of ammonia-N: phosphate-P excretion was 11·3:1. Total Kjeldahl-nitrogen level of *S. hispida* was found to be 6·23 µg A N/mg dry weight. Thus, the average excretion of ammonia-N over a 24-hour period was approximately 14·5% of the total Kjeldahl-nitrogen content.

References

COOPER, L. H. N., 1937. "The nitrogen cycle in the sea". J. mar. biol. Ass. U. K., **22**: 183–204.

CUSHING, D. H., 1954. "Some problems in the production of oceanic plankton". Document VIII presented to the Commonwealth Oceanographic Conference, 1954. (Not seen by author.)

DAVID, P. M., 1955. "The distribution of *Sagitta gazellae* Ritter-Zahony". Discovery Rep., **27**: 235–78.

DUGDALE, R. C., & DUGDALE, V. A., 1962. "Nitrate and ammonia uptake in the Sargossa Sea". Unpublished MS. Bermuda Biol. Sta. – Atomic Energy Commission Rept. Contr. AT (30–1)–2646. Sept. 1, 1962.

GARDINER, A. C., 1937. "Phosphate production by planktonic animals". J. Cons. int. Explor. Mer, **12**: 144–46.

GUILLARD, R. R. L., 1963. "Organic sources of nitrogen for marine centric diatoms". *In*: "Symposium on marine microbiology" (Carl H. OPPENHEIMER, ed.) Charles C. Thomas. Springfield, Illinois. pp. 93–104.

HARRIS, E., 1959. "The nitrogen cycle in Long Island Sound". Bull. Bingham oceanogr. Coll., **17**: 31–65.

HYMAN, L. H., 1959. "The Invertebrates: Vol. V. Smaller Coelomate Groups". McGraw-Hill Book Co., Inc., New York. pp. 1–71.

KETCHUM, B. H., 1962. "Regeneration of nutrients by zooplankton". Rapp. Cons. Explor. Mer, **153**: 142–47.

KRUSE, J., & MELLON, M. G., 1952. "Colorimetric determination of free ammonia with a pyridine-pyrazolone reagent". Sewage Ind. Wastes, **24**: 1098–1100.

MARSHALL, S. M., & ORR, A. P., 1958. "Some uses of antibiotics in physiological experiments in sea water". J. Mar. Res., **17**: 341–46.

MARSHALL, S. M., & ORR, A. P., 1961. "On the biology of *Calanus finmarchicus*. XII. The phosphorus cycle: excretion, egg production, autolysis". J. mar. biol. Ass. U. K., **41**: 463–88.

MENZEL, D. W., & RYTHER, J. H., 1961. "Seasonal variations in the abundance and composition of zooplankton in the Sargasso Sea off Bermuda, 1960–1961". Unpublished MS. Bermuda Biol. Sta. – Atomic Energy Commission Rept. Contr. AT(30–1)–2646. June 1, 1961.

MENZEL, D. W., & SPAETH, J. P., 1962. "The occurrence of ammonia in Sargossa Sea waters and in rain water at Bermuda." Limnol. Oceanogr., **7**: 159–62.

MULLIN, J. B., & RILEY, J. P., 1955. "The spectrophotometric determination of nitrate in natural waters, with particular reference to sea water". Anal. Chem. Acta, **12**: 464–80.

MURPHY, J., & RILEY, J. P., 1958. "A single-solution method for the determination of soluble phosphate in sea water". J. mar. biol. Ass. U. K., **37**: 9–14.

PARRY, D. A., 1944. "Structure and function of the gut in *Spadella cephaloptera* and *Sagitta setosa*". J. mar. biol. Ass. U. K., **26**: 16–36.

PERRIN, C. H., 1953. "Rapid modified procedure for determination of Kjeldahl nitrogen". Anal. Chem., **25**: 968–71.

PIERCE, E. L., 1951. "The Chaetognatha of the West Coast of Florida". Biol. Bull. Woods Hole, **100**: 206–28.

POMEROY, L. R., MATHEWS, H. M., & MIN, H. S., 1963. "Excretion of phosphate and soluble organic phosphorus compounds by zooplankton". Limnol. Oceanogr., **8**: 50–55.

PROSSER, C. L., & BROWN, F. A., Jr., 1961. "Comparative Animal Physiology". W. B. Saunders Co., Philadelphia. 688 pp.

RIGLER, F. H., 1961. "The uptake and release of inorganic phosphorus by *Daphnia magna* Straus". Limnol. Oceanogr., **6**: 165–74.

VACCARO, R. F., 1961. "The oxidation of ammonia in sea water". J. Cons. int. Explor. Mer, **27**: 3–14.

ZOBELL, C. E., 1935. "The assimilation of ammonium nitrogen by *Nitschia closterium* and other marine phytoplankton". Proc. Nat. Acad. Sci., **21**: 517–22.