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Oceanic mixotrophic flatworms*

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ABSTRACT: Most reports of photosynthetic flatworms are from benthic or littoral habitats, but small (< 1 mm) acoel flatworms with algal endosymbionts are a widespread, though sporadic, component of the open-ocean plankton in warm waters. Among oceanic flatworms are specimens harboring prasinophyte or, less commonly, dinophyte endosymbionts. Photosynthesis was measured by ^{14}C uptake in flatworms from shelf/slope waters in the western north Atlantic and from the Sargasso Sea. Rates were as high as 27 ng C fixed ind.$^{-1}$ h^{-1} Assimilation ratios ranged from 0.9 to 1.3 ng C fixed (ng Chlorophyll *a*)$^{-1}$ h^{-1} Although these acoels were photosynthetic, they were also predatory on other plankton. Remains of crustaceans and radiolarian central capsules were observed in the guts or fecal material of some specimens. These acoel-algal associations apparently depend on both autotrophic and heterotrophic nutrition and are thus mixotrophic. Among the planktonic protozoa, mixotrophy is a common nutritional strategy, it also appears to be common strategy among certain taxa of open-ocean metazoa.

INTRODUCTION

Acoel flatworms (class Turbellaria, order Acoela) are common inhabitants of the marine littoral environment. While reports of pelagic acoels are rare (Brauner 1920, Hyman 1939, Dörjes 1970, Bush 1984), we have found, through special collecting and preservation techniques, that small (< 1 mm) acoels are a common, but sporadic, component of the plankton in the upper water column in warm, oceanic waters. These as-yet undescribed species can be abundant but, considering the rarity with which they are reported, they apparently are overlooked in most plankton samples.

During the past 13 yr, we have collected incidental data on the occurrence and distribution of acoels in surface waters of the open-ocean. All of the species we have collected harbor algal endosymbionts. Algal symbiosis is well known among some temperate water, littoral acoels, such as *Convoluta roscoffensis* and *C. convoluta* (Keeble 1910, Ax & Apelt 1965, Taylor 1971, Mettan 1979, Bush 1984, Smith & Douglas 1987) and are especially common among tropical, littoral species of *Convoluta* (Antonius 1968). Endosymbionts also appear in the majority of the described warm-water, planktonic species, specifically *Haplodiscus* spp., *C. pelagica*, *C. schultzei*, and *Adenopea illardatus* (Löhner & Micoletzky 1911, Dörjes 1970).

Planktonic acoels are not unique among oceanic zooplankton in being mixotrophic. Ciliates, radiolarians, foraminiferans, and acantharians with algal chloroplasts or endosymbionts are common in surface waters and are thought to be important in open-ocean plankton communities as sites of enhanced primary production and as grazers and predators (Anderson 1983, Swanberg 1983, Spero & Parker 1985, Michaels 1988b, Stoecker in press). Because of their size (usually > than ~64 µm), both mixotrophic planktonic acoels and sarcodines should contribute disportionately for their biomass and primary production to the flux of sinking particles out of the mixed layer (Michaels & Silver 1988).

We document the widespread occurrence of small, mixotrophic acoels in warm, oceanic waters, present data on their algal endosymbionts, chlorophyll contents and gut contents, and give experimental data on the photosynthetic rates of these planktonic acoel-algal associations.

METHODS AND MATERIALS

Acoels were observed and collected by SCUBA divers in conjunction with other work on various cruises in the Sargasso Sea, the eastern Atlantic (vicin-

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ity of the Madeira and Canary Islands) and in the Indian Ocean (vicinity of the Seychelles) (Table 1 and Fig. 1). In slope waters and in the Sargasso Sea, acoels were also collected with a 64 µm mesh, 0.5 m diam. net in the top 3 m of the water column (Table 1). The net was deployed during calm weather to windward while

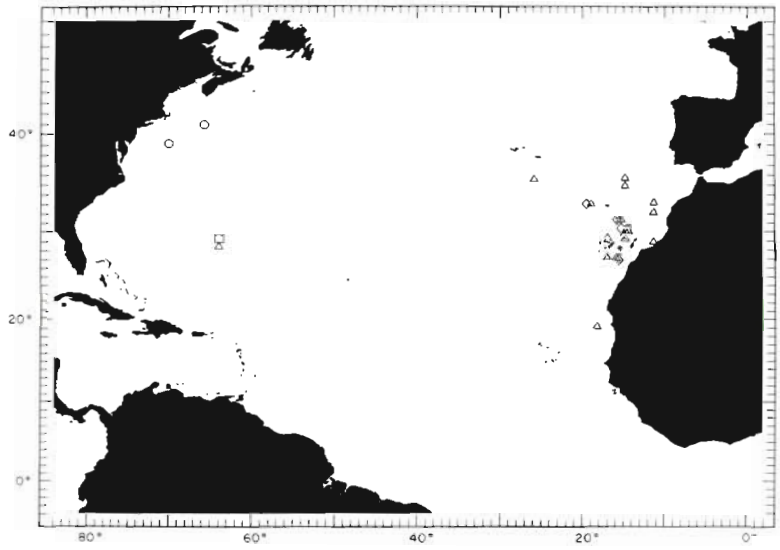


Fig. 1. Stations in the North Atlantic where mixotrophic acoel flatworms were collected. ○: *Convoluta* sp. 'A'; □: unidentified species 'B'; △: unidentified species 'C'; ◇: unidentified species 'D'. See Table 1 for further information

Table 1 Observations of planktonic acoel flatworms with algal endosymbionts in the open ocean

Position	Date	Temp (°C)	Taxon	Size (mm)	Color
ATLANTIC OCEAN					
Slope Waters, NW					
39° N, 70° W	31 Aug–2 Sep 1986	24	*Convoluta* sp. 'A'	0.6–0.9	Bright green[a]
41° N, 66° W	3 Jul 1987	18	*Convoluta* sp. 'A'	0.6–0.9	Bright green[a]
Sargasso Sea					
29° N, 64° W	24–30 May 1988	25	Unidentified sp. 'B'	0.3–0.5	Golden[a]
28° N, 64° W	22 Nov 1975	23	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
Eastern Atlantic					
36° N, 26° W	9 Jul 1978	21	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
36° N, 15° W	30 Oct 1978	21	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
35° N, 15° W	30 Oct 1978	21	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
33° N, 11° W	31 Oct 1978	21	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
32° N, 11° W	31 Oct 1978	21	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
30° N, 15° W	1 Nov 1978	22	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
29° N, 15° W	1 Nov 1978	23	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
29° N, 17° W	2 Nov 1978	23	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
28° N, 11° W	2 Nov 1978	23	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
27° N, 17° W	3 Nov 1978	24	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
19° N, 18° W	6 Nov 1978	25	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
31° N, 16° W	25 May 1986	19	Unidentified sp. 'D'	< 1.0	Bright green[b]
			Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
30° N, 15° W	26 May 1986	19	Unidentified sp. 'D'	< 1.0	Bright green[b]
			Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
27° N, 16° W	30 May 1986	19	Unidentified sp. 'D'	< 1.0	Bright green[b]
			Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
33° N, 19° W	4 June 1986	19	Unidentified sp. 'D'	< 1.0	Bright green[b]
			Unidentified sp. 'C'	0.5–1.0	Dark brown[b]
INDIAN OCEAN					
2° S, 55° E	21 Apr 1976	28	Unidentified sp. 'C'	0.5–1.0	Dark brown[b]

[a] Collected with a 64 µm mesh, 0.5 m diam. plankton net between 0 and 4 m
[b] Collected by hand with SCUBA in upper 100 m

the ship was allowed to drift. Specimens were also collected off the Canary Islands with a 150 μm mesh, 0.7 m diam. net in short vertical hauls.

Freshly collected specimens were checked at sea for the presence of algal endosymbionts; wet mounts were made of small, semi-transparent specimens but smears were made of the larger, more opaque specimens. These preparations were then examined with transmitted light and epifluorescence microscopy (a Zeiss microscope equipped with a 50 W Mercury lamp and a BP 450–490 nm excitation filter, an LP 520 nm barrier filter, and an FT 510 nm chromatic beam splitter). Endosymbionts appeared as pigmented bodies with transmitted light and under epifluorescent illumination they had a red fluorescence, indicating the presence of chlorophyll *a* (Chl *a*).

Specimens were preserved for later examination with light and transmission electron microscopy (TEM). The best results were obtained when the acoels were anesthetized in $MgCl_2$ isotonic with ambient seawater for 5 min prior to fixation (Smith & Tyler 1984). Good preservations for light and EM were obtained when specimens were fixed in 3 % buffered glutaraldehyde (0.1 M sodium cacodylate in 90 % seawater, pH 7.2) and stored at 4 °C. Some anesthetized acoels were fixed in unbuffered glutaraldehyde drawn under a glass coverslip which was used to restrain the specimen. Fixation in Bouin's, a recommended method for acoels (Smith & Tyler 1984), was tried with the slope water specimens (Table 1); they disintegrated in this fixative.

For light microscopy, acoels were embedded in Epon, serially sectioned at 2 μm section thickness, and stained with alcian blue, hematoxylin and eosin (Smith & Tyler 1984). For TEM, some specimens were post-fixed in 1 % osmium tetroxide, stained with 0.5 % uranyl acetate, dehydrated in an ethanol series, and then embedded in Spurr's low-viscosity resin or in Epon. Some specimens were post-fixed in osmium and embedded in LX-112 without prestaining. After sectioning, the specimens were stained with uranyl acetate and lead citrate and viewed at 60 kV on a Zeiss 10CA EM or on a Philips 201 EM.

In 1986 to 1988 some specimens were kept alive and transported to the laboratory where they were maintained for several weeks. Specimens collected in slope waters in 1986 (Table 1) were held at room temperature (~ 23 °C) in GF/F filtered seawater in polycarbonate containers. They were exposed to cool-white fluorescent light at 125 $\mu E\ m^{-2}\ s^{-1}$ PAR for 14 h d^{-1}. Specimens collected from slope waters in 1987 (Table 1) were kept at 15 °C under approximately the same light regime. Small amounts of zooplankton (mostly *Acartia tonsa*) were provided as prey. The worms were transferred to fresh, GF/F filtered seawater every 3 to 5 d.

The chlorophyll content of specimens collected from slope waters in 1987 and from the Sargasso Sea in 1988 was determined fluorometrically (Parsons et al. 1984). One to 5 individuals were rinsed in filtered seawater, and dropped onto each replicate GF/F filter. The filters with the acoels were frozen and later ground in 90 % acetone to extract the chlorophyll.

Photosynthesis was measured by uptake of ^{14}C in specimens collected from slope waters in 1986 and 1987 and from the Sargasso Sea in 1988 (Table 1). Individuals were transferred through 2 washes of GF/F filtered seawater prior to incubation in GF/F filtered seawater with an added ca 0.5 $\mu Ci\ ml^{-1}$ ^{14}C bicarbonate (specific activity, 53 mCi $mmol^{-1}$; New England Nuclear). Triplicate 100 μl samples of the spiked seawater were taken for determination of total activity in the media. (Incubation conditions varied among experiments and are given in Table and Fig. legends.)

In 1986 and 1987, the samples were prepared for liquid scintillation counting by transferring individuals into scintillation vials and then adding 2 ml of Scintigest (Fisher Scientific) to kill worms and digest their tissues. After 12 h at room temperature, 2 ml of 5 % acetic acid in methanol were added to each vial and the vials were evaporated to dryness (this step removed the inorganic ^{14}C). Then, 1 ml of distilled water was added to each vial, followed by 10 ml of scintillation fluid (ScintiVerse II, Fisher Scientific).

Sample preparation in 1988 was modified so that data on chlorophyll content and ^{14}C uptake could be obtained from the same specimens. After incubation, 5 worms were placed on a GF/C glassfiber filter and frozen. Later, the filters were ground in 5 ml of 90 % acetone and chlorophyll was measured fluorometrically. The acetone extract and sedimented debris were added to a scintillation vial and allowed to evaporate to dryness at 60 °C. Two ml of 5 % acetic acid in methanol were added, and the vials were evaporated to dryness again. Sample processing then proceeded as described above.

Samples were counted on a Packard Tri-Carb 4000 Series liquid scintillation counter (United Technologies Packard) using the external standard ratio to correct for quench. The uptake of ^{14}C due to photosynthesis was estimated by subtracting the average uptake in the dark controls from uptake in the light. Rates of photosynthesis were calculated from light-mediated ^{14}C uptake as described in Parsons et al. (1984).

RESULTS AND DISCUSSION

We have observed at least 3 acoel species in warm (usually > 20 °C) oceanic waters (Table 1 and Fig. 1). Bright green acoels with prasinophyte endosymbionts

(*Convoluta* sp. 'A') were observed in the western Atlantic. Chlorophyll contents and photosynthetic rates were measured. Specimens of a similar worm (unidentified species 'D') were collected in the eastern Atlantic. A small, golden acoel (unidentified sp. 'B') with a dinophyte endosymbiont was collected in the Sargasso Sea. Chlorophyll content and photosynthetic rates were measured in the golden acoel. A third type (unidentified species 'C'), a dark brown acoel associated with gelatinous zooplankton, was collected in both the Atlantic and Indian Oceans. A prasinophyte endosymbiont was documented to occur in this acoel, but no data were collected on the chlorophyll contents or photosynthetic rates in this acoel-algal association.

Bright green acoels

Among the free-swimming acoels in surface waters, the most common species is a small (ca 0.9 mm in length when mature) undescribed species of *Convoluta* (*Convoluta* sp. 'A' in Table 1). This species is usually a bright, grass green color, although in most populations a few less-intensely pigmented individuals occur. Sexually mature individuals were collected southeast of Georges Bank in July, 1987 (Table 1). Light-histological sections of these specimens showed that they belong in the species group containing *C. convoluta, C. sordida,* etc. (Group 4 in Fig. 80 of Antonius 1968); i.e., they had a ciliated male antrum. They did not, however, match a known species description. We have collected small, bright green acoels which we believe to be the same, or a closely related species, from the eastern Atlantic (unidentified sp. 'D' in Table 1). Small, unidentified, bright green, free-living flatworms have also been reported from the Indian (Norris 1967) and Pacific Oceans (Michaels 1988b).

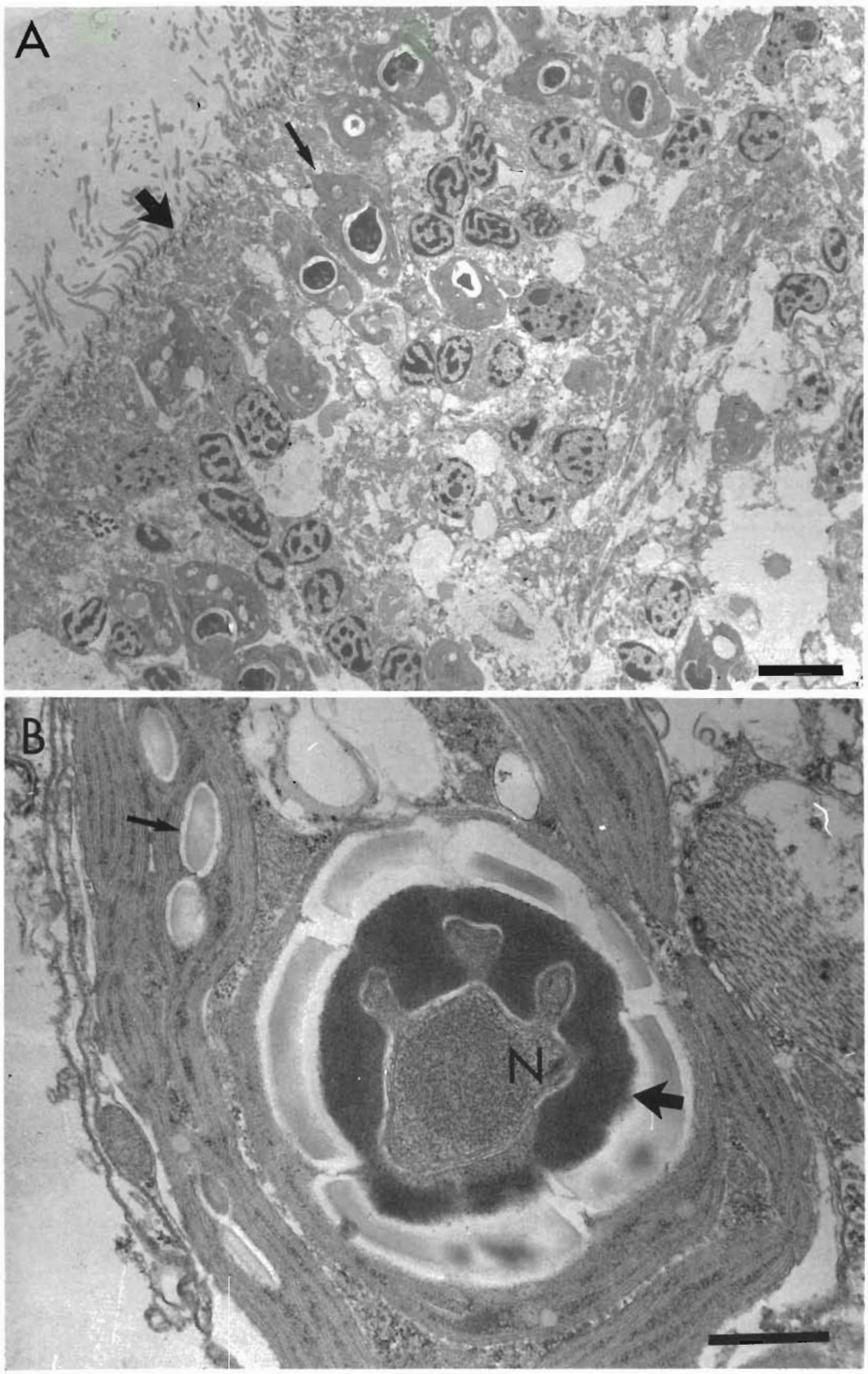
In slope waters and in the eastern Atlantic when we have encountered them, the bright green acoels were patchy in distribution. Swarms sometimes reach densities of an estimated 10 to 100 m^3. In the Canary Islands in 1986, short vertical hauls collected several hundred bright green worms and divers reported that the worms occurred at densities of 10's to 100's m^{-3} in a narrow zone. It is possible that *Convoluta* sp. 'A' or similar species are widespread in warm oceanic waters, but further sampling and taxonomic study are necessary before this can be confirmed.

TEM has been used to identify the algal endosymbionts in specimens of *Convoluta* sp. 'A' collected in 1986 and 1987 (Table 1). Based on their ultrastructure (Figs. 2 and 3) the algae are members of the green line; they have a large central pyrenoid, starch is stored in plates around the pyrenoid and between the thylakoids, and the thylakoids are somewhat randomly arranged with inter-connections between the grana. We were able to further identify the algae as *Tetraselnis* sp. subgenus *Prasinocladia* sp. (family Prasinophyceae) based on the unique ultrastructure of the pyrenoid (Fig. 2a, b); in this subgenus protrusions of the nucleus enter the pyrenoid (Hori et al. 1983). Prasinophyte endosymbionts occur in *C. roscoffensis* (Keeble & Gamble 1907, Oschman & Gray 1965, Gooday 1970, Holligan & Gooday 1975, Douglas 1983, 1985) and *C. psammophila* (Sarfatti & Bedini 1965), 2 littoral species. The endosymbiotic algal cells in our specimens lacked thecae and flagella; loss of these organelles also occurs in endosymbionts of *C. roscoffensis* (Douglas 1983). The endosymbionts tended to be concentrated just under the body wall (Fig. 2A) ostensibly lying in cells of the peripheral of central parenchyma. In *C. roscoffensis,* the symbionts are often closely associated with muscle fibers of the host (Oschman 1966). The same appears true of our *Convoluta* sp. 'A' (Fig. 3).

Photosynthesis was measured in *Convoluta* sp. 'A' collected at both locations in the western North Atlantic. The specimens from over Alvin Canyon (39°N 70°W; 24°C) fixed ca 28 ng C ind.$^{-1}$ h^{-1} at an irradiance of 125 µE m^{-2} s^{-1} (Table 2). Rates of photosynthesis in individuals collected in 1987 were less than 50 % of the rates in individuals collected in 1986 and incubated at the same irradiance (Table 2). Even at irradiances which were light-saturating, rates of photosynthesis in worms from 1987 were only a ca 30 % of the rate determined at 125 µE m^{-2} s^{-1} in 1986. It is possible that the observed differences on the 2 occasions were due to differences in temperature or incubation conditions (refer to Table 2) or due to the physiological state of the worms and/or their endosymbionts. The specimens collected southeast of Georges Bank were sexually mature whereas those collected in 1986 were not.

Chlorophyll measurements are only available for the mature worms collected in 1987; they contained an average of 7.5 ng chl *a* ind.$^{-1}$ when collected; 18 d later their chl *a* content was about the same and the assimilation ratio was ca 1.2 ng C (ng chl *a*)$^{-1}$ h^{-1} at a saturat-

Fig. 2. *Convoluta* sp. 'A' collected over Alvin Canyon (39°N 70°W; see Table 1). A: Algal endosymbionts (thin arrow) are concentrated under the body wall (thick arrow) of the flatworm. Scale bar = 5 µm. B: The endosymbiont can be identified as a *Tetraselmis* sp. based on its chloroplast structure. Starch grains (thin arrow) lie between the thylakoids and a starch shell surrounds the core of the pyrenoid (thick arrow). The protrusion of the algal nucleus (N) into the pyrenoid is characteristic of the subgenus Prasinocladia (Hori et al. 1983). Scale bar = 1 µm


A
B
N

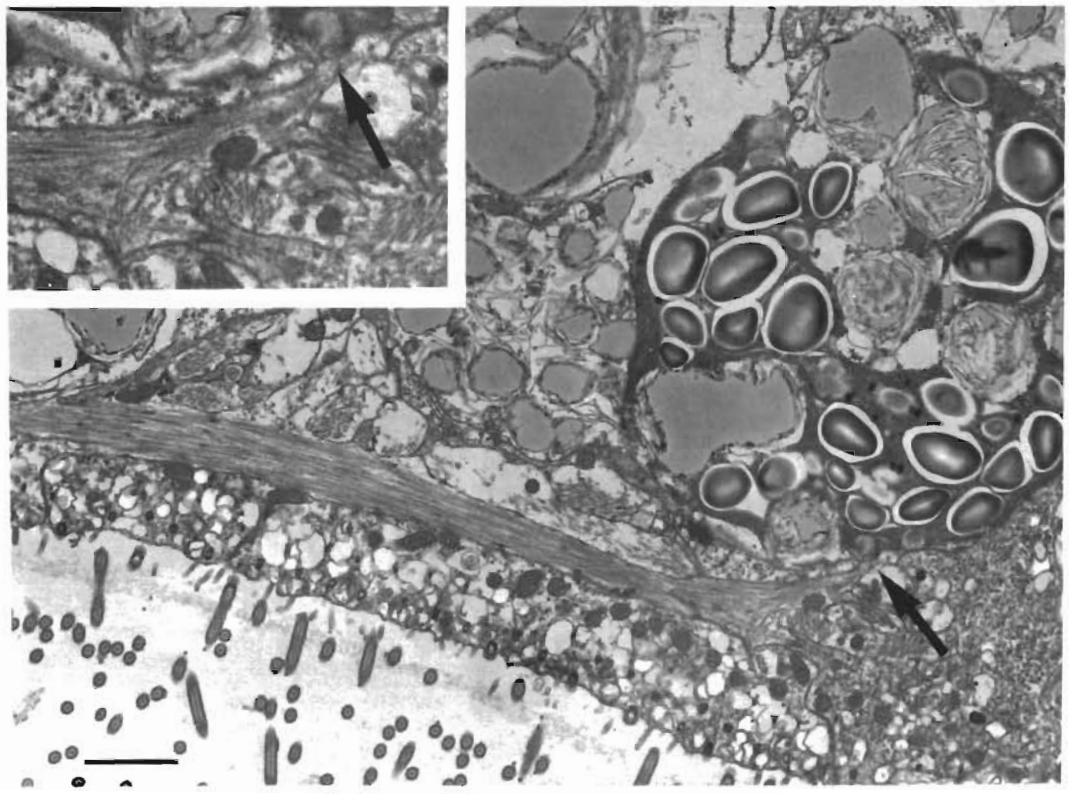


Fig. 3. *Convoluta* sp. 'A' collected south of Georges Bank (41° N, 66° W; see Table 1). The algal cell lies close to a muscle fiber (the point of contact is indicated by the arrow). This contact is shown at a higher magnification in the inset. Note the numerous starch grains in the alga (also a *Tetraselmis* sp.). The ciliated epithelium of the flatworm is shown at the bottom of the micrograph. Scale bar = 1 µm

Table 2. *Convoluta* sp. 'A' Chlorophyll *a* contents and photosynthetic rates. Means ± SD

Year	Site	ng chl *a* ind.$^{-1}$	Irradiance (µE m^{-2} s^{-1})	ng C fixed ind.$^{-1}$ h^{-1}
1986	39° N, 70° W	•	125	27.7 ± 4.5 (N = 6)[a]
1987	41° N, 66° W	7.5 ± 3.8 (N = 5)	100	11.2 ± 1.7 (N = 13)[b]
1987	41° N, 66° W	6.8 ± 4.9 (N = 5)	100	0.6 ± 0.2 (N = 6)[c]
			250	6.0 ± 1.3 (N = 6)
			500	8.7 ± 2.4 (N = 6)
			1000	7.7 ± 2.7 (N = 5)
			1500	8.0 ± 4.3 (N = 5)

• no data
[a] 12 d post-collection; 1 h incubation at 23 °C
[b] 1 d post-collection; 6 h incubation at 18 °C
[c] 18 d post-collection; 6 h incubation at 20 °C; assimilation ratio: ~ 1.2 ng C (chl *a*)$^{-1}$ h^{-1}

ing irradiance (Table 2). This is similar to what might be expected in late exponential/early stationary phase free-living phytoplankton (Glover 1980).

Crustacean cuticle, along with other unidentifiable material, was present in the central syncytium (where digestion occurs) of the mature *Convoluta* sp. 'A' obtained in 1987 (Fig. 4). When we maintained *Convoluta* 'A' in the laboratory, we observed that copepods added to their culture containers disappeared. In the dark or dim light, these acoels aggregated on top of maimed copepods and appeared to be browsing on them. This behavior ceased immediately if lights were turned on. We do not know how the worms capture intact copepods. In the field, the worms swim freely, turning frequently in what could be an effective search pattern. Many of the littoral acoels with symbionts prey

on small invertebrates (Sarfatti & Bedini 1965, Taylor 1971) although *C. roscoffensis* stops feeding once it acquires algal endosymbionts (Keeble 1910, Holligan & Gooday 1975).

Golden acoel

During 1988, we collected a second type of small acoel with symbionts (unidentified sp. 'B' in Table 1). This type was a semi-transparent golden color. All the specimens we collected were immature and thus none could be identified. We observed golden-brown gymnodinoid cells ca 18 to 22 µm in length in the worms. The mean number of symbionts ind. $^{-1}$ was 103 (SD = 36, n = 21). TEM confirmed our field identification of the endosymbiont as a dinoflagellate (Fig. 5) based on the mesokaryotic nucleus characteristic of this family and on chloroplast structure (Dodge 1979). We have tentatively identified the dinophyte as a *Symbiodinium* sp. based on its symbiotic habit, gymnodinoid shape, and peripheral lobed chloroplast. Dinoflagellate endosymbionts have been reported in littoral, tropical acoels; *Amphiscolops* usually has an *Amphidinium* endosymbiont but *Haplodiscus* can have both *Amphidinium* and *Symbiodinium* endosymbionts (Taylor 1971, Trench & Winsor 1987). However, the *Symbiodinium* sp. in our acoel does not have a stalked pyrenoid like that found in the *Symbiodinium* found in *Haplodiscus* by Trench & Winsor (1987).

On-deck ^{14}C incubations were done on 2 dates with the golden acoels from the Sargasso Sea. The acoels collected on May 25, 1988 had an average chl *a* content of 2.5 ng ind.$^{-1}$ (SD 0.7, n = 21) and a maximum rate of photosynthesis (P_{max}) of 2.5 ng C ind.$^{-1}$ h^{-1}, SD = 0.6 [1.0 ng C (ng chl *a*)$^{-1}$ h^{-1}] (Fig. 6A). Worms collected on May 27 had a lower chl *a* content, 1.6 ng ind.$^{-1}$ (SD = 1.0, n = 21), a lower photosynthetic rate ind^{-1} (P_{max} 1.4 ng ind^{-1} h^{-1}, SD = 0.0) and a lower chlorophyll specific rate of photosynthesis [~ 0.9 ng C (ng chl *a*)$^{-1}$ h^{-1}] (Fig. 6B). We suspect that these differences between the 2 experiments reflect differences in the physiological state of the acoel/algal associations collected on the 2 d. However, as has been observed in symbiotic foraminiferans (Spero & Parker 1985), there was little or no photoinhibition in either experiment (Fig. 6); this suggests that the acoel/algal associations are adapted to high-light such as occurs at the sea surface.

Dark brown acoel

The third type of acoel flatworm with algal symbionts that we observed was a dark brown species ca 0.5 to 1.0 mm in length (unidentified sp. 'C' in Table 1; also

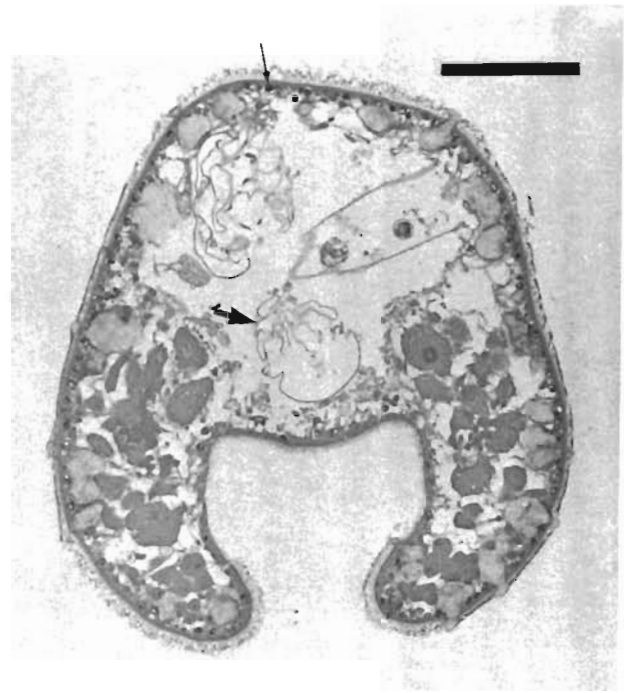


Fig. 4. *Convoluta* sp. 'A' collected south of Georges Bank (41° N 66° W; see Table 1). Light micrograph of a cross-section stained with alcian blue and hematoxylin and eosin. Algal endosymbionts (thin arrow) are located under the epidermis; copepod cuticle (thick arrow) and other remnants are visible in the central syncytium. Scale bar = 100 µm

see Swanberg 1979). This species has been observed mostly on the surfaces of colonial radiolarians and other gelatinous zooplankton. Divers have rarely observed free-swimming individuals. Acoel 'C' has been observed in association with colonial radiolarians in the Sargasso Sea, the eastern Atlantic, and the Indian Ocean (Table 1). None of our preserved specimens were sexually mature or the genitalia were not intact; therefore it has not been possible to identify this type to species but the presence of a single bursal mouth-piece in 1 sectioned specimen is consistent with being a species of *Convoluta*.

Freshly collected specimens of acoel 'C' deposited yellowish fecal material that contained radiolarian central capsules as well as other unidentifiable material (Swanberg 1979). It seems likely that this acoel preys on colonial radiolarians or consumes injured radiolarian tissues. It is also possible that it scavenges prey captured by the radiolarians or by symbionts associated with them.

Tissue smears of fresh material indicated that acoel 'C' contained algal endosymbionts but TEM was necessary to identify the symbiont as a prasinophyte (Fig. 7). Identifying characteristics were the large central pyrenoid surrounded by a starch shell, the thylakoid arrangement, and protusion of the nucleus into the pyrenoid (Dodge 1979). No data are available on the chlorophyll content or rates of photosynthesis in acoel 'C'

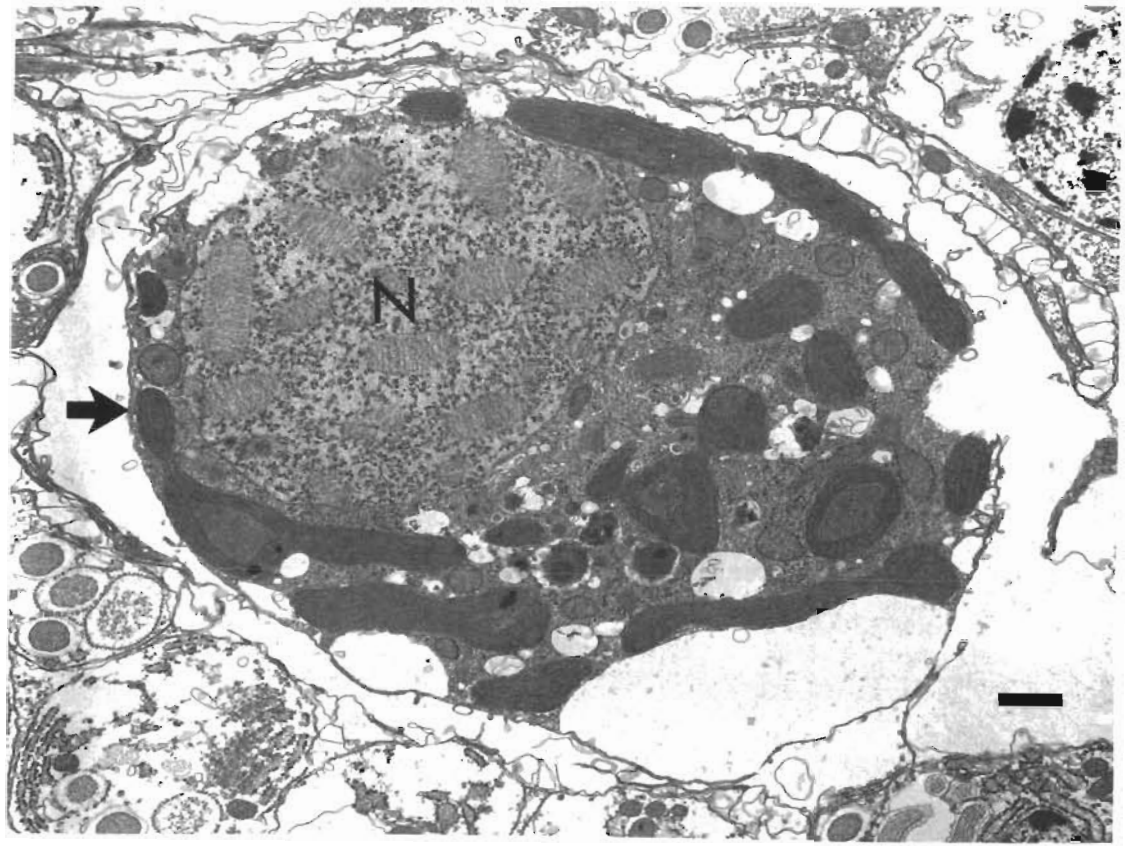


Fig. 5. Dinophyte endosymbiont (arrow) in acoel sp. 'B' (see Table 1). The mesokaryon nucleus (N) characteristic of dinophytes is evident. Scale bar = 1 µm. Note the peripheral, lobed chloroplast

CONCLUSION

Photosynthetic, mixotrophic acoel turbellarians are a widespread component of the open-ocean plankton in warm waters. These metazoans have been overlooked in most studies of oceanic plankton, probably because they become unrecognizable with the fixation procedures routinely used for plankton samples (they contract into a tight sphere if they are not anesthezed prior to fixation).

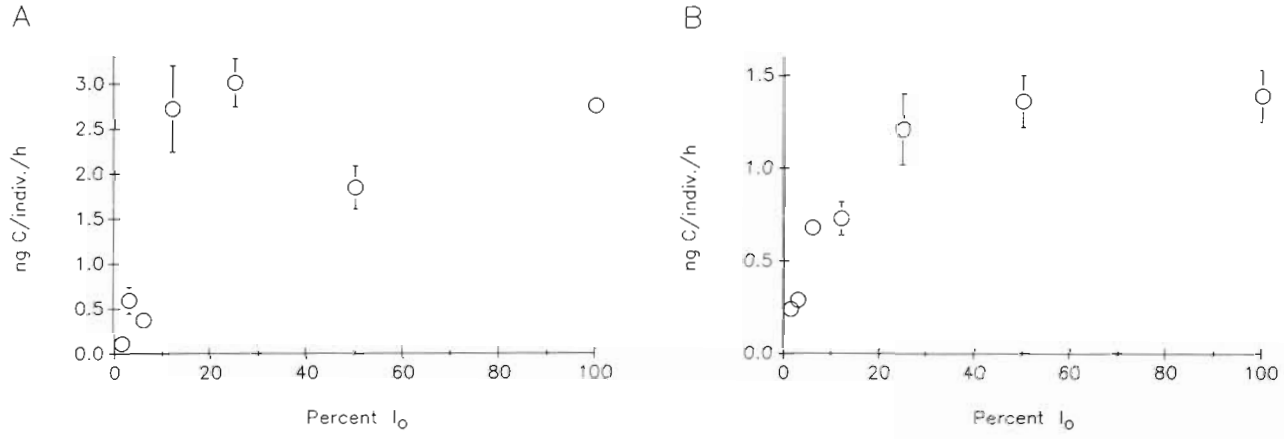


Fig. 6. Photosynthesis vs irradiance curves for acoel sp. 'B' (see Table 1). A: on May 25 (I° = 2507 µE m^{-2} s^{-1}); B: May 27 (I° = 1976 µE m^{-2} s^{-1}). Specimens were incubated during mid-day for 2 h in an on-deck flow-through incubator; seawater temperature was 25 °C. In the first incubation (A), the mean chl *a* content was 2.5 (SD = 0.7) ng ind.$^{-1}$ and the assimilation ratio was at 1.0 ng C (ng chl *a*)$^{-1}$ h^{-1} at saturating irradiance (average for 4 highest light levels). In the second incubation (B), the chlorophyll content was 1.6 (SD = 1.0) ng ind.$^{-1}$ and the assimilation ratio at saturating irradiance (average for 3 highest light levels) was 0.9 ng C (ng chl *a*)$^{-1}$ h^{-1}

Large (> 64 μm) mixotrophic organisms appear to be common in warm oceans. Among the large protozoan mixotrophs are the colonial radiolarians (Swanberg 1983), many solitary radiolarians (reviewed in Anderson 1983), and many planktonic foraminiferans (reviewed in Taylor 1982, Spero & Parker 1985, Gastrich & Bartha 1988), and acantharians (Michaels 1988a, b). Our data show that mixotrophic metazoans may also be important in the open ocean. Some of the planktonic hydromedusae and scyphomedusae also have algal endosymbionts (reviewed in Taylor 1984, Muscatine et al. 1986, Trench 1987, Kremer et al. unpubl.). Although each of these types of mixotroph probably makes only a small contribution to the total biomass and chlorophyll (Swanberg 1983, Michaels 1988a), as an assemblage, large mixotrophs may be quite important in oligotrophic oceans (Caron & Swanberg 1989).

Photosynthetic mixotrophs often have high photosynthetic rates per unit chlorophyll and are thought to be extremely efficient utilizers of both carbon and nitrogen derived from prey; thus, they may be more important in carbon and inorganic nutrient cycling than their biomass alone would suggest (Taylor 1982, Anderson 1983, Michaels 1988a). The assimilation ratios, 0.9 to 1.2 ng C fixed (ng chl a)$^{-1}$ h^{-1}, that we observed in the acoels were about 10-fold lower than those that have been observed in planktonic radiolarians with algal symbionts (Swanberg 1983, Rivkin & Lessard 1986). Nevertheless, acoels, like colonial radiolarians (Swanberg 1983), should be important as localized, microscale sites of high primary productivity. These symbiotic associations are also important because of their size; they are large compared with most phytoplankton. They should contribute disproportionately for their biomass to the flux of energy and materials out of the mixed layer (Michaels & Silver 1988). The ecology of metazoan as well as protozoan associations with endosymbiotic algae needs to be studied in the open ocean.

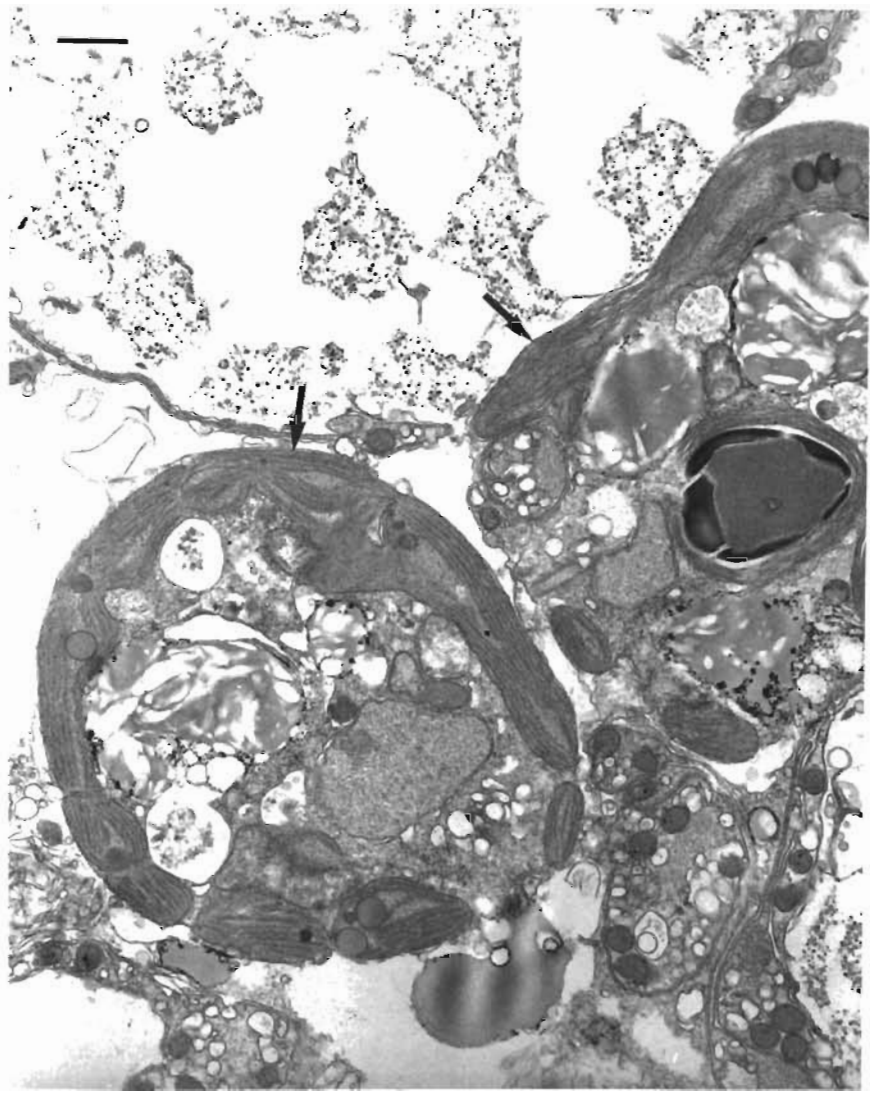


Fig. 7. Prasinophyte endosymbionts (arrows) in acoel sp. 'C' collected in the vicinity of the Canary Islands (30° N 15° W; see Table 1). Note the large central pyrenoid surrounded by a starch shell in the algal cell on the right. A protusion of the nucleus can be seen in the pyrenoid. Scale bar = 1 μm

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