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Spatial distribution of symbiont-bearing dinoflagellates in the Indian Ocean in relation to oceanographic regimes

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ABSTRACT: The spatial distribution of symbiont-bearing dinoflagellates was investigated during a cruise from Cape Town, South Africa to Broome, Australia. Endo- and ectosymbionts were only found in the order Dinophysiales. The genera *Ornithocercus*, *Histioneis*, *Parahistioneis* and *Citharistes* had cyanobacteria as ectosymbionts, while the genera *Amphisolenia* and *Triposolenia* contained both intact cyanobacterial and eukaryotic endosymbionts. The symbiont-bearing dinoflagellates were mainly found in the upper 100 m of the water column. Their distribution was restricted to water temperatures exceeding 16.5°C, and the highest species diversity and cell concentrations were found at temperatures around 20 to 30°C. The symbiont-bearing dinoflagellates were always associated with water masses with low nutrient (N-limited) and chl *a* concentrations. Special attention was given to the ectosymbiont-bearing dinoflagellates. Under light microscopy, some of the food vacuoles of *Ornithocercus* spp. resembled ectosymbionts in size, shape and colour. Transmission electron microscopy of *O. magnificus* and *O. quadratus* revealed the presence of a peduncle and many rhabdosomes; both may serve in prey capture. Also, numerous food vacuoles were present, but their content was generally too degraded for a proper identification of prey type. However, occasionally remnants of eukaryotes were observed, indicating that *Ornithocercus* spp. may feed on ciliates. Thus, our data suggest that the ectosymbiont-bearing dinoflagellates use a multi-resource strategy (photosynthesis and phagotrophy) to cope with a low-nutrient environment.

KEY WORDS: Symbionts · *Ornithocercus* · *Amphisolenia* · *Histioneis* · Dinophysoids · Dinoflagellates · Indian Ocean · Galathea 3

INTRODUCTION

The world's oceans are estimated to contribute around half of global net primary productivity, with approximately one-quarter of this attributed to oligotrophic regions (Field et al. 1998). These oligotrophic areas are dominated by small primary producers (Li 1995, Maranon et al. 2003), in particular cyanobacteria. However, some of these small primary producers also form symbioses with other organisms. Symbiosis among protists varies from a relatively loose attachment of one species to another (ectosymbiosis) to an intimate endocytic relationship with one symbiont living within the protoplasm of the other (Norris 1967).

Heterotrophic dinoflagellates of the order Dinophysiales, e.g. the genera *Histioneis*, *Ornithocercus*, *Parahistioneis*, *Amphisolenia* and *Triposolenia* are known to form symbioses with cyanobacteria and eukaryotes (Kofoid & Skogsberg 1928, Lucas 1991, Gordon et al. 1994, Janson et al. 1995, Jyothibabu et al. 2006). These genera contain very conspicuous species, with cell lengths of up to several hundreds of micrometres. Usually the symbionts are ectosymbionts, either associated with the cingular groove or found in

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special cavities of the dinoflagellate cell body (Norris 1967), but in some cases the symbionts are intracellular, such as the symbionts of *Amphisolenia* spp. (Lucas 1991).

Symbiont-bearing dinoflagellates (here and throughout the text meaning species of Dinophysiales) have been found in tropical to subtropical regions, and they occur almost exclusively in oligotrophic oceans such as the Pacific (Gómez 2005, Foster et al. 2006a,b), Indian (Wood 1963a, Taylor 1976) and Atlantic Oceans (Foster et al. 2006a,b). Although they probably have a wide distribution in subtropical and tropical seas, very few quantitative data are available on their distribution in time and space. Their apparent restriction to nitrogen-limited oligotrophic tropical oceans have led some authors to suggest that the photosynthetic symbionts are able to fix N_2, and the host may benefit from this (Gordon et al. 1994, Jyothibabu et al. 2006). Because only bacteria are known to fix N_2, this explanation only holds true for those species which have cyanobacterial symbionts, unless of course N_2-fixing heterotrophic bacteria are involved (Farnelid & Riemann 2008). Cyanobacterial symbionts have been found in species belonging to the genera *Amphisolenia*, *Histioneis*, *Ornithocercus* and *Parahistioneis*. However, nitrogenase activity (seen with immunolabelling-transmission electron microscopy [TEM] using nitrogenase antisera) has so far only been found in one type of cyanobacteria, a symbiont of *H. depressa* (Foster et al. 2006a).

It is still unknown whether and how the symbiont-bearing dinoflagellates utilise photosynthetic products from the cyanobacterial symbionts. Do they utilise dissolved organic matter leaking from the cyanobacteria or do they ingest some of their symbionts and thereby gain organic matter? Food vacuoles have been observed inside the dinoflagellate hosts (Lucas 1991). In the case of *Ornithocercus magnificus*, *Histioneis dolon* and *Parahistioneis para*, food vacuoles and remnants of cyanobacterial symbionts have been observed inside their food vacuoles (Lucas 1991).

The aim of this investigation was to (1) investigate the distribution of symbiont-bearing dinoflagellates across the southern Indian Ocean in relation to oceanographic regimes, qualitatively and quantitatively, and (2) examine *Ornithocercus* spp. under light microscopy and TEM to study food vacuole content and feeding apparatus and search for possible prey-capture cell organelles to understand their role in the marine pelagic ecosystem.

MATERIALS AND METHODS

Sample location and environmental measurements. Samples were taken from aboard the Danish Navy surveillance frigate 'F359 Vædderen' during Leg 7 of the 3rd Danish Galathea expeditions around the world during 2006–2007. Leg 7 went from Cape Town in South Africa to Broome in northwestern Australia in the period 18 October to 16 November 2006. We present data from 21 stations across the Indian Ocean and along a transect perpendicular to Broome in northwestern Australia (Fig. 1, Table 1). The Seabird 9/11 CTD and a light sensor mounted on a rosette equipped with twelve 30 l Niskin bottles were used to measure oceanographic parameters, including irradiance, salinity and temperature. The vertical CTD profiles were repeated thrice to a depth of at least 400 m along the

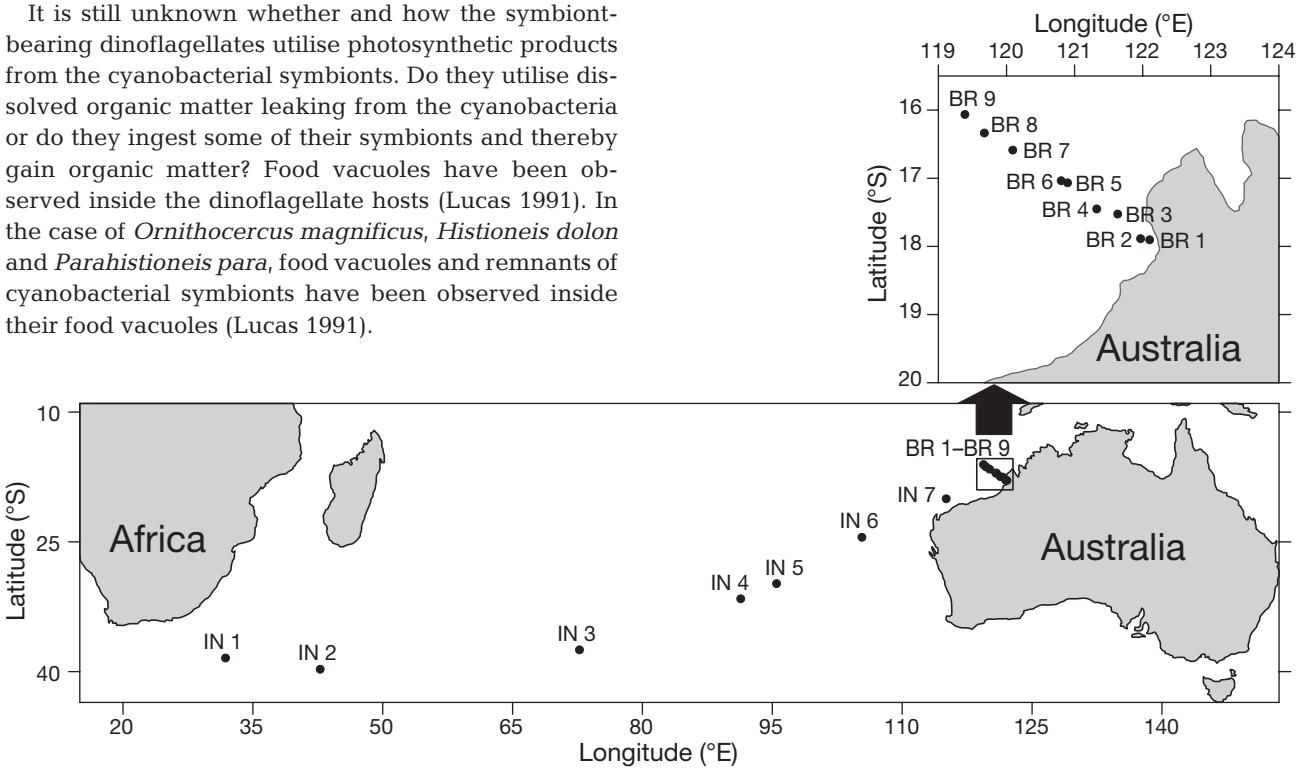

Fig. 1. Location of sampling stations along the Indian Ocean (Stns IN1 to IN7) and Broome (Stns BR1 to BR9) transects

Table 1. Position and maximum depth of sampling stations. nd: no data

Stn	Latitude (°S)	Longitude (°E)	Maximum depth (m)
Indian Ocean			
IN1	38° 29′	31° 43′	4002
IN2	39° 33′	42° 46′	2430
IN3	37° 16′	72° 30′	nd
IN4	31° 24′	91° 11′	4355
IN5	29° 35′	95° 15′	2747
IN6	24° 27′	105° 14′	5303
IN7	19° 46′	114° 51′	1381
Broome			
BR1	17° 46′	121° 52′	39
BR2	17° 41′	121° 44′	103
BR3	17° 28′	121° 27′	195
BR4	17° 17′	121° 08′	198
BR5	17° 03′	120° 49′	170
BR6	16° 50′	120° 34′	358
BR7	16° 26′	119° 56′	675
BR8	16° 15′	119° 38′	1142
BR9	16° 01′	119° 19′	1852

Indian Ocean transect and lower depth in some stations along the Broome transect depending on their maximum depth at each station, starting at 08:00 h, with downward cast used to describe the water column structure and water samples taken during each upward cast. Nutrients and chlorophyll *a* (chl *a*) concentrations were determined from the first CTD cast at 08:00 h.

Light: Light attenuation was estimated from photosynthetically active radiation (PAR) data collected with the CTD measured relative to a surface PAR sensor (Biospherical QSR). Data were fitted to a 2-phase exponential decay equation:

$$E(z) = ref \times \exp(-K \times z) \text{ for depth} \leq z_{\mathbf{shift}} \quad (1)$$
$$E(z) = ref \times \exp(-K_{\mathbf{d1}} \times z_{\mathbf{shift}}) \times \exp(-K_{\mathbf{d2}} \times (z - z_{\mathbf{shift}})$$

where $E(z)$ is irradiance at the depth z, K_{d1} and K_{d2} the diffuse attenuation coefficients above and below the depth $z_{\mathbf{shift}}$, respectively, and *ref* is reflection from the surface. All 4 parameters (*ref*, $K_{\mathbf{d1}}$, $K_{\mathbf{d2}}$, $z_{\mathbf{shift}}$) were fitted to data from each CTD cast in 1 step using SAS software (1990) after data corrupted by wave actions and instrument failure were omitted.

Nutrients: Samples of 33 ml for determination of nutrient concentration were taken directly from the water bottle and frozen immediately after sampling. The nutrient concentrations were later analysed at the National Environmental Research Institute (NERI) in an automatic nutrient analyser (Dansk Havteknik) following Grasshoff (1976). Nutrient samples were analysed with a detection limit of 0.06, 0.1, 0.04, 0.3 and 0.2 µM for phosphorus, nitrate, nitrite, ammonia and silicate, respectively.

Pigments: Chl *a* and phaeopigments (phaeo) concentrations (total and >10 µm) were measured on 500 and 1000 ml triplicate samples filtered onto Whatman GF/F and 10 µm filters respectively. The filters were extracted overnight in 5 ml of 96% ethanol (Jespersen & Christoffersen 1987) and measured before and after acid addition (3 drops of 1 N HCl) on a Turner Designs Model 700 fluorometer calibrated against a pure chl *a* standard. Chl *a* values were converted to carbon assuming a C:chl *a* ratio of 50.

The oceanographic parameters, including irradiance, salinity, temperature, nutrient concentrations and chl *a* concentration, are reported in detail in A. W. Visser et al. (unpubl.), but used here in the analysis of the observed distribution patterns of symbiont-bearing dinoflagellates.

Identification and enumeration of symbiont-bearing dinoflagellates. Water samples (10 l) were collected from each discrete depth (4 to 7 depths) within the upper 400 m using 30 l Niskin bottles arranged on a CTD rosette. Then water samples were immediately concentrated using 20 µm mesh-size plankton net in the laboratory. The concentrated samples were preserved in 1% neutral Lugol's solution and subsequently filtered onto a 0.2 µm black polycarbonate filter. When approximately 1 ml sample was left in the chimney, 0.2 ml Calcofluor White M2R (Polysciences; 10 mg l^{-1} dissolved in distilled water) was added, and the filtration was continued after 5 min until the filter was dry. The dry filter was mounted on immersion oil (Type DF, Cargille Laboratories) on a slide. Another drop of immersion oil was then placed on the filter and a cover glass mounted on the top of the immersion oil (Andersen & Kristensen 1995). This sample slide was used for species identification and enumeration of symbiont-bearing dinoflagellates by epifluorescence microscopy, using UV excitation (330 to 385 nm) and an appropriate emission filter (420 nm) (using an Olympus BX50 microscope fitted with Olympus DP70 digital camera). Dinoflagellates were identified using Kofoid (1906, 1907), Kofoid & Michener (1911), Kofoid & Skogsberg (1928), Wood (1954, 1963a,b), Abé (1967) and Taylor (1976). Live plankton samples were collected using a 20 µm mesh-size plankton net and vertical hauls from about 70 m depth to the surface. Samples were examined and selected cells were photographed shortly after sampling using an Olympus BX51 microscope with a Soft-Imaging ColorView III digital camera.

Correlation between symbiont-bearing dinoflagellates and selected environmental variables were investigated using Pearson's correlation analysis (SPSS Statistics 17.0 software). Prior to the analysis, normality (Kolmogorov-Smirnov test) and homogeneity (Levene's test) were tested and when necessary, the data were log (x+1)-transformed.

Fine structure of *Ornithocercus magnificus* and *O. quadratus*. Cells (*Ornithocercus magnificus* and *O. quadratus*) for TEM observations were single cells isolated from live plankton samples collected 31 October 2007 at Stn IN5 and fixed in 2% glutaraldehyde in 0.05 M Na-cacodylate buffer with 0.25 M sucrose (final concentrations) for 1.5 h. After a brief rinse in sterile filtered seawater, cells were individually transferred to a small drop of warm 1.5% agar made up in seawater. The hardened agar drops were then washed in 0.1 M Na-cacodylate buffer with different sucrose concentrations: 0.25 M and 0.125 M sucrose and lastly in straight buffer. The drops were transferred every 20 min and finally fixed for 1 h in 2% OsO_4. After a brief rinse in buffer, cells were dehydrated in a graded ethanol series and embedded in Spurr's resin via propylene oxide. The material was sectioned on a Reichert Ultracut E ultramicrotome using a diamond knife, and the sections were collected on slot grids and placed on Formvar film. After staining in uranyl acetate and lead citrate, sections were examined in a JEOL JEM-1010 electron microscope operated at 80 kV. Micrographs were taken using a GATAN 792 digital camera. A total of 9 cells were examined (7 *O. magnificus* and 2 *O. quadratus*).

RESULTS

Hydrography and chlorophyll *a*

Indian Ocean transect (Stns IN1 to IN7)

The temperature in the surface waters ranged from 13.7°C at the southernmost station, IN2, on the South African coast, to 25.9°C at Stn IN7 on the west coast of Australia (Fig. 2A). Surface salinity was in the range of 34.9 to 35.9. The euphotic zone (i.e. irradiance above 1% of that at the surface) ranged between 25.5 and 102 m along the Indian Ocean transect (Fig. 2A). Inorganic nitrogen concentration variability in surface water was large, from 3.1 to 3.9 µmol l^{-1} at Stns IN2 and IN3 to <0.1 µmol l^{-1} at Stns IN4 to IN7, indicating a likely nitrogen limitation of the surface waters in this area (0 to 10 m). Phosphate concentrations ranged from 0.1 to 0.4 µmol l^{-1}. Similarly, maximum concentrations of phosphate were found at Stns IN2 and IN3 (0.4 µmol l^{-1}), while much lower concentrations (<0.10 µmol l^{-1}) were found at Stns IN6 and IN7. The N:P ratio in surface water was between 0.13 and 13, lower than the Redfield ratio of 16:1, further supporting the idea of nitrogen as the

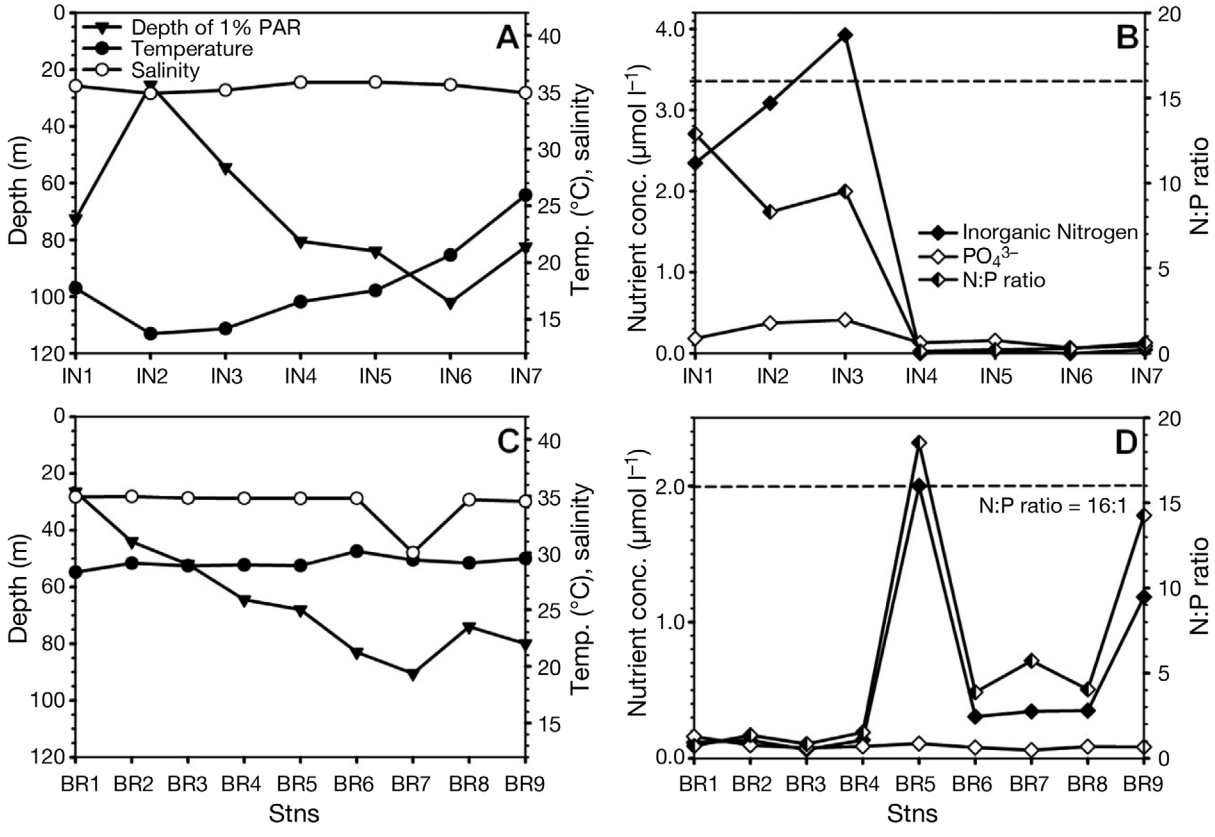

Fig. 2. Spatial distribution of environmental parameters (temperature, salinity, depth of 1% photosynthetically active radiation [PAR]) and nutrients (inorganic nitrogen, PO_4^{3-}, N:P ratio) in surface waters (10 m) at each station in the (A,B) Indian Ocean and (C,D) Broome transects. Dashed line: Redfield ratio of C:N = 16. Note the different scales for nutrient concentration in (B) & (D)

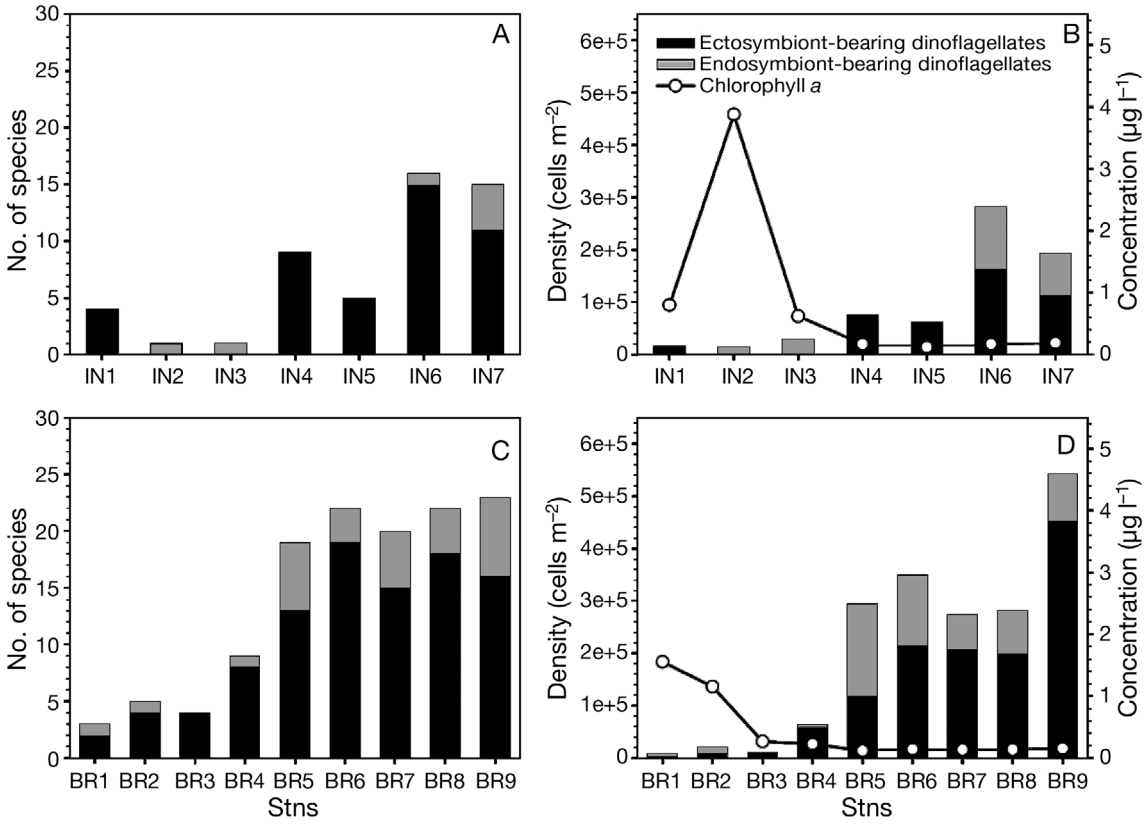

Fig. 3. (A,C) Number of species of symbiont-bearing dinoflagellates and (B,D) integrated densities of symbiont-bearing dinoflagellates and chl *a* concentration in the (A,B) Indian Ocean and (C,D) Broome transects

limiting nutrient (Fig. 2B). Chl *a* concentrations in surface water along this transect ranged from 0.1 to 3.9 µg chl *a* l^{-1}; the highest concentration was found at the coastal stations (Fig. 3B).

Broome transect (Stns BR1 to BR9)

Surface temperatures varied from 28.3 to 30.1°C, while salinity ranged from 34.5 to 35, except for Stn BR7, where the salinity was only 30.0 (Fig. 2C). The depth of the euphotic zone varied from 27 m close to the coast to 91 m offshore (Fig. 2C). The concentration of inorganic nitrogen varied from 0.06 to 2 µmol l^{-1}, with the highest concentrations at Stns BR5 and BR9 (Fig. 2D) in the upper 10 m. Phosphate concentrations varied from 0.06 to 0.16 µmol l^{-1} in the upper 10 m, with the highest concentration found at the coastal station (Stn BR1). The N:P ratio in surface water was between 0.7 to 5.7 with the exception of very high N:P ratios of 14.3 and 18.5 at Stns BR9 and BR5, respectively. Chl *a* concentrations in surface waters along this cruise transect varied between 0.1 and 1.6 µg chl *a* l^{-1}. The highest chl *a* concentrations were found at the coastal stations (Fig. 3D).

Species diversity

A total of 45 species of symbiont-bearing dinoflagellates were found on this cruise. Most of these (33 species) had cyanobacterial ectosymbionts and belonged to 4 genera of family Dinophysiaceae: *Ornithocercus*, *Citharistes*, *Histioneis* and *Parahistioneis*. The remaining 12 species had eukaryotic or prokaryotic endosymbionts and belonged to the genera *Amphisolenia* and *Triposolenia* within the family Amphisoleniaceae (Table 2).

The largest diversity of symbiont-bearing dinoflagellates was found within the genera *Histioneis* and *Amphisolenia*, with 23 and 10 species observed, respectively (Table 2). The most common ectosymbiont-bearing dinoflagellate species were *H. striata* Kofoid & Michener 1911, and *Ornithocercus heteroporus* Kofoid 1907, both found at 12 stations. Other common species were *O. magnificus* Stein, 1883 and *H. depressa* Schiller 1933, found at 11 and 10 stations, respectively. Many species of ectosymbiont-bearing dinoflagellates were rare, however, and only encountered once at a single station, like *H. biremis* Stein 1883, *H. dolon* Murray & Whitting 1899, *H. panda* Kofoid & Michener 1911, *H. paulseni* Kofoid 1907, *H.*

Table 2. Abundance of symbiont-bearing dinoflagellates in the Indian Ocean (both transects) and the surface temperature range of their occurrence

Species	Cell density (cells l^{-1})	Surface temperature (°C)
Ectosymbiont-bearing dinoflagellates		
Citharistes regius Stein 1883[a]	0.1–0.6	20.7–30.1
Histioneis biremis Stein 1883[b]	0.1	30.1
H. carinata Kofoid 1907	0.1	29.1–29.5
H. depressa Schiller 1933	0.1–0.7	17.7–30.1
H. dolon Murray & Whitting 1899	0.1	29.1
H. elongata Kofoid & Michener 1911	0.1–0.3	17.5–30.1
H. hippoperoides Kofoid & Michener 1911	0.1–0.4	16.5–30.1
H. hyalina Kofoid & Michener 1911	0.1	16.5–28.9
H. inclinata Kofoid & Michener 1911	0.1–0.2	16.5–30.1
H. joergensenii Schiller 1928[a]	0.1	20.7–25.9
H. longicollis Kofoid 1907	20.1	0.7–30.10
H. mitchellana Murray & Whitting 1899	0.1–0.2	17.5–30.1
H. oxypteris Schiller 1928[a]	0.1–0.3	20.7–30.1
H. pacifica Kofoid & Skogsberg 1928[b]	0.1–0.2	16.5–29.5
H. panaria Kofoid & Skogsberg 1928	0.1	25.9–30.1
H. panda Kofoid & Michener 1911	0.1	29.1
H. paulseni Kofoid 1907	0.1	20.7
H. reginella Kofoid & Michener 1911[b]	0.1	30.1
H. reticulata Kofoid 1907	0.1	16.5–29.4
H. rotundata Kofoid & Michener 1911	0.3	29.1–30.1
H. schilleri Böhm 1931	0.2	29.4
H. striata Kofoid & Michener 1911[b]	0.1–0.6	16.5–30.1
H. tubifera Böhm 1931	0.1	29.1
H. vouki Schiller 1928	0.1	28.9
Ornithocercus carpentariae Wood 1963[a]	0.1–0.2	20.7–30.1
O. heteroporus Kofoid 1907	0.1–2.0	16.5–30.1
O. magnificus Stein 1883	0.1–0.6	16.5–30.1
O. quadratus Schütt 1900	0.1–0.5	16.5–30.1
O. splendidus Schütt 1895	0.1	30.1
O. steinii Schütt 1900	0.1–0.2	20.7–29.1
O. thumii Schmidt 1888	0.1–0.4	20.7–30.1
Parahistioneis para Murray & Whitting 1899	0.1	28.9–29.5
P. paraformis Kofoid & Skogsberg 1928	0.1–0.3	25.9–29.5
Endosymbiont-bearing dinoflagellates		
Amphisolenia bidentata Schröder 1900	0.1–0.8	25.9–30.1
A. brevicauda Kofoid 1907	0.1	28.9–29.5
A. elongata Kofoid 1907[b]	0.1	29.5
A. globifera Stein 1883	0.1–1.2	13.7–30.1
A. inflata Murray & Whitting 1899[b]	0.1	25.9–30.1
A. laticincta Kofoid 1907	0.1	29.4
A. palmata Stein 1883	0.1–0.2	28.9–29.5
A. rectangulata Kofoid 1907	0.1	29.1–29.5
A. schauinslandi Lemmermann 1899	0.1	28.9
A. thrinax Schütt 1893	0.1	29.4
Triposolenia bicornis Kofoid 1906	0.3–0.5	25.9–28.9
T. truncata Kofoid 1906[b]	0.1	29.1

[a]First record in the Indian Ocean
[b]First record in the seas off northwestern Australia

reginella Kofoid & Michener 1911, *H. tubifera* Böhm 1931, *H. schilleri* Böhm 1931, *H. vouki* Schiller 1928 and *O. splendidus* Schütt 1895.

The most common endosymbiont-bearing dinoflagellate species were *Amphisolenia bidentata* Schröder 1900 and *A. globifera* Stein 1883, both observed at 9 stations, while *A. brevicaudata* Kofoid 1907, *A. elongata* Kofoid 1907, *A. laticincta* Kofoid 1907, *A. schauinslandi* Lemmermann 1899, *A. thrinax* Schütt 1893 and *Triposolenia truncata* Kofoid 1906 were each only encountered once at a single station.

The number of symbiont-bearing dinoflagellate species was quite low (≤4 species) at Stns IN1 to IN3 (closest to South Africa). The number of species increased along the Indian Ocean transect and the highest numbers of species were found at Stns IN6 and IN7 (15 to 16 species; Fig. 3A). On the Broome transect, the lowest numbers of symbiont-bearing dinoflagellate species (3 to 5 species) were found at the coastal stations (Stns BR1 to BR3), while up to 23 species of symbiont-bearing dinoflagellates were found at Stn BR9 (Fig. 3C).

Horizontal and vertical distributions

Ectosymbiont-bearing dinoflagellates

The abundances of ectosymbiont-bearing dinoflagellates ranged from the detection limit of 0.1 to 1.5 cells l^{-1} on the Indian Ocean transect (Fig. 4A). The highest cell concentrations were found in the upper 100 m, but cells were in some cases still present at 200 to 400 m depths. Depth-integrated abundances (0 to 400 m) were in the range of 0 to 1.6×10^5 cells m^{-2}, clearly showing the lowest cell densities at Stns IN1 to IN3 and the highest at Stns IN6 and IN7 (Fig. 3B).

Cell densities of ectosymbiont-bearing dinoflagellates on the Broome transect were in the range 0.1 to 4.0 cells l^{-1} (Fig. 4C). The coastal stations (Stns BR1 to BR4) had the lowest cell densities, while considerably higher densities were found near the shelf (Stns BR5 and BR6). The highest cell densities were found in the upper 100 m, but cells were also present at 400 m depth. The integrated densities were in the range of 4.6×10^3 to 4.5×10^5 cells m^{-2} (0 to 400 m; Fig. 3D). The outermost station (Stn BR9) had the highest density per area, while the innermost station had the lowest.

Physical and chemical parameters, i.e. irradiance, temperature and inorganic nutrients, changed considerably along the transects, which may have influenced

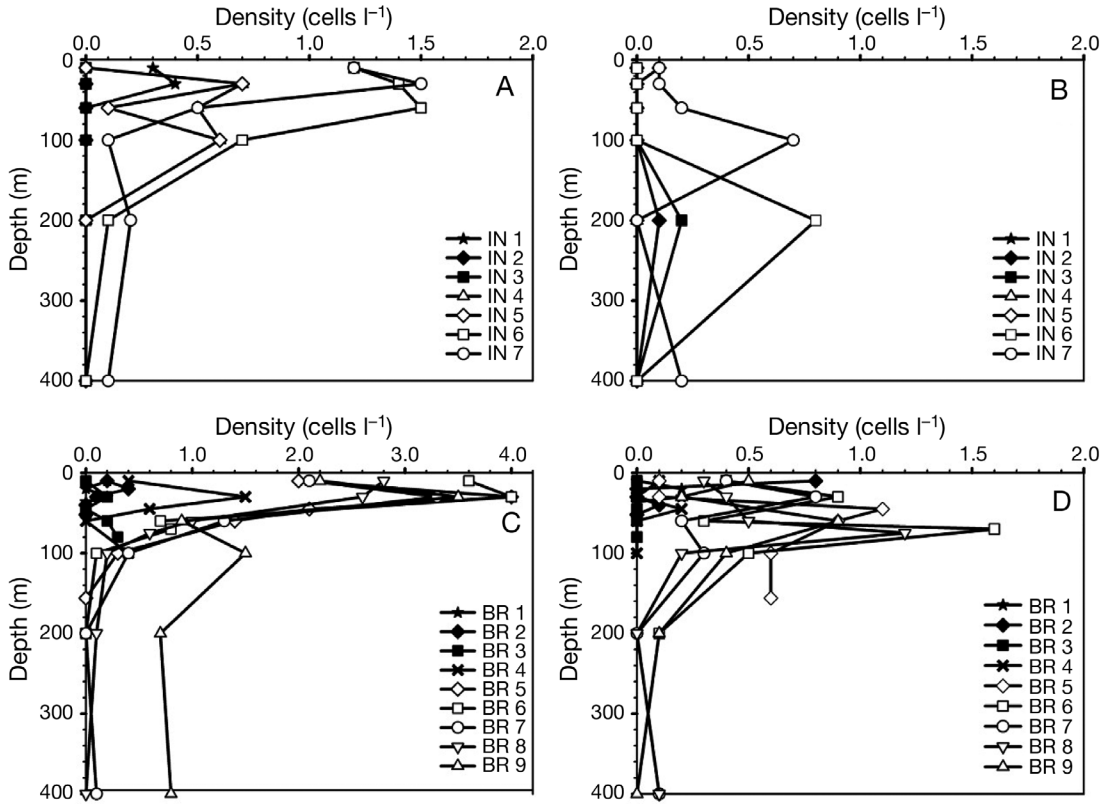

Fig. 4. Vertical distribution of (A,C) ectosymbiont-bearing dinoflagellates and (B,D) endosymbiont-bearing dinoflagellates in the (A,B) Indian Ocean and (C,D) Broome transects. Note the different scale in (C)

the distribution of the organisms. The highest cell densities were found in the euphotic zone, but cells were also found in the aphotic zone, where they received very low irradiances (Fig. 5A). Likewise, the highest cell densities were found at water temperatures >20°C, and cell concentrations clearly increased with temperature (Fig. 5B, Table 3), though some cells were still present at temperatures of 16 to 20°C, and a few cells were even found at temperatures <16°C. In accordance with these observations, correlation analyses revealed that cell densities of ectosymbiont-bearing dinoflagellates were positively correlated to irradiance and temperature (Table 3).

In the euphotic zone, nearly all ectosymbiont-bearing dinoflagellates were found at inorganic nitrogen concentrations of <2 µmol l^{-1} (Fig. 5C) and the highest cell densities (3.0 to 4.0 cells l^{-1}) were recorded at inorganic nitrogen concentrations <0.5 µmol l^{-1}. Despite this, no significant correlation was found between inorganic nitrogen concentration and cell density in the euphotic zone. Taking only data from the euphotic zone with temperatures >16°C into consideration, the correlation between cell density and inorganic nitrogen was still not statistically significant. In the aphotic zone, the inorganic nitrogen concentration varied much more and reached concentrations as high as 28 µmol l^{-1}; yet the correlation between cell densities and the concentration of inorganic nitrogen was not significant (Fig. 5D, Table 3).

In the euphotic zone, the vast majority of ectosymbiont-bearing dinoflagellates were found at PO_4^{3-} concentrations <0.50 µmol l^{-1}(Fig. 5E), and the highest cell densities were found at stations which also had very low PO_4^{3-} concentrations (<0.10 µmol l^{-1}; Fig. 5E). In accordance with this, a significant negative correlation between cell densities and inorganic phosphorous was observed in the euphotic zone (Table 3). In the aphotic zone, the concentrations of PO_4^{3-} varied more and concentrations as high as 2 µmol l^{-1} were found (Fig. 5F). However, the correlation between cell density and PO_4^{3-} concentration in the aphotic zone was not significant (Fig. 5F, Table 3).

Endosymbiont-bearing dinoflagellates

Abundances of endosymbiont-bearing dinoflagellates were very low along the Indian Ocean transect and in some cases (Stns IN1, IN4 and IN5), even absent (Figs. 3B & 4B). The abundance ranged from the detection limit of 0.1 to 0.8 cells l^{-1}. Highest concentrations were generally found between 100 and 200 m depth,

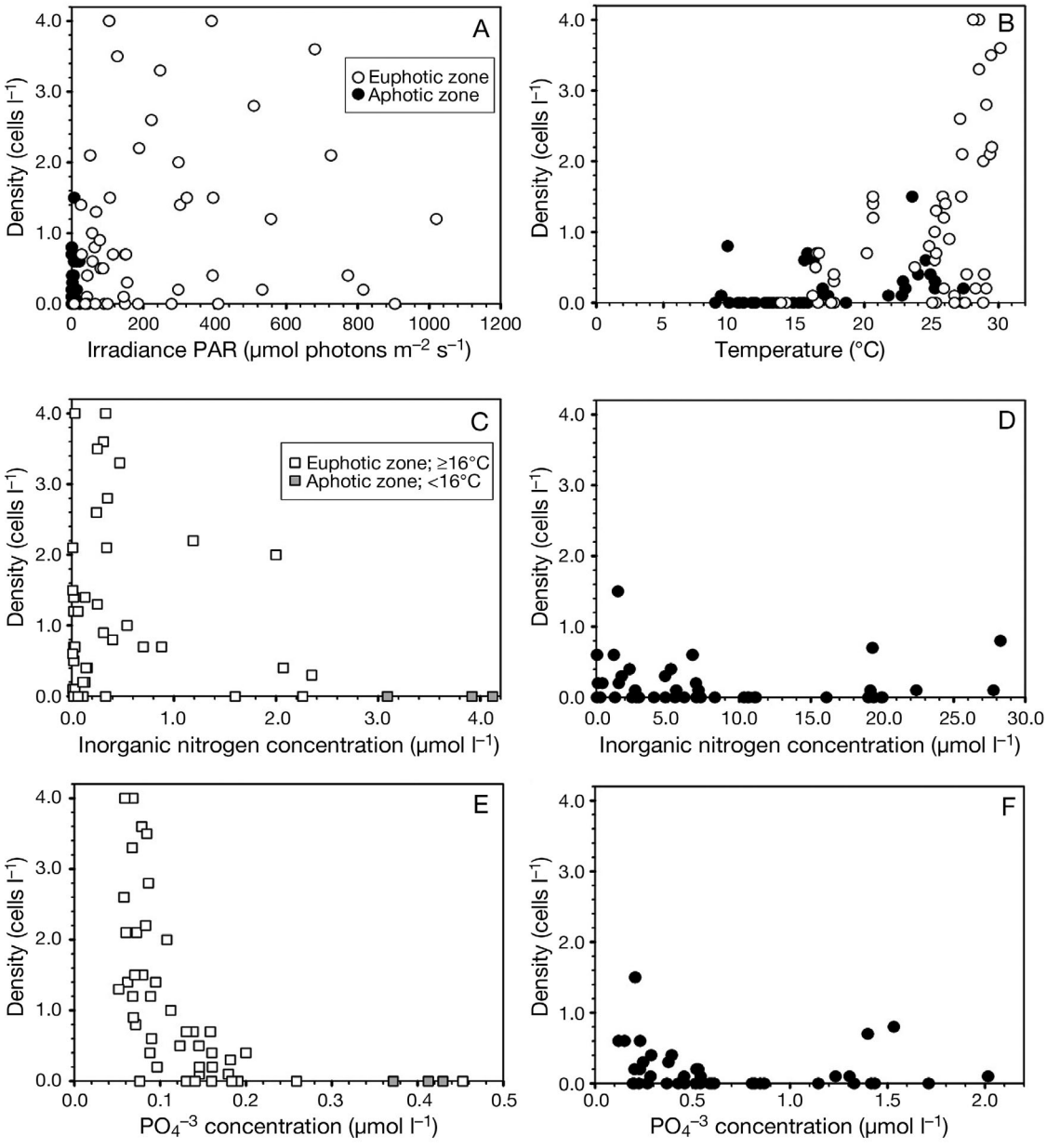

Fig. 5. Ectosymbiont-bearing dinoflagellates. Relationship between cell density and (A) irradiance; (B) temperature; inorganic nitrogen concentration in the (C) euphotic zone and (D) aphotic zone; and PO_4^{3-} concentration in the (E) euphotic zone and (F) aphotic zone. PAR = photosynthetically active radiation

Table 3. Pearson's correlation coefficient (r) between density of symbiont-bearing dinoflagellates and selected physical and chemical variables. Significance evaluated at 95% confidence level ($p \leq 0.05$). **$p < 0.01$. ns: not significant; n: number of data points

Variable	Density of ectosymbiont-bearing dinoflagellates				Density of endosymbiont-bearing dinoflagellates		
	All data r (n = 92)	Euphotic zone r (n = 49)	Euphotic zone[a] r (n = 46)	Aphotic zone r (n = 43)	All data r (n = 92)	Euphotic zone r (n = 49)	Aphotic zone r (n = 43)
Temperature	0.548**				0.407**		
Irradiance	0.539**				0.158 (ns)		
Inorganic nitrogen		−0.224 (ns)	−0.031 (ns)	−0.238 (ns)		−0.127 (ns)	−0.108 (ns)
PO_4^{3-}		−0.649**	−0.647**	−0.199 (ns)		−0.437**	−0.153 (ns)

[a]only from the euphotic zone with temperatures >16°C

indicating a subsurface maximum, but cells were recorded down to 400 m (Fig. 4B). Depth integrated (0 to 400 m) cell densities were between 1.5×10^4 and 1.2×10^5 cells m^{-2}, with the highest values found at Stns IN6 and IN7 (Fig. 3B).

Cell concentrations of endosymbiont-bearing dinoflagellates ranged from 0 to 1.6 cells l^{-1} along the Broome transect (Fig. 4D). Very low abundances were found at Stns BR1, BR3 and BR4 (<0.2 cells l^{-1}), while the highest cell concentrations were found at Stn BR6 (at 70 m; Fig. 4D). The highest cells densities were always found in the upper 100 m depth of the water column. However, some cells still occurred in deeper water (Fig. 4D). The density per unit area ranged from below the detection limit to 1.77×10^5 cells m^{-2}, with lowest concentrations at Stns BR1 to BR4 and highest at Stns BR5 (Fig. 3D).

Taking all the data into consideration, endosymbiont-bearing dinoflagellate abundances were not significantly correlated to irradiance (Table 3, Fig. 6A). Abundances were highest at temperatures >22°C and

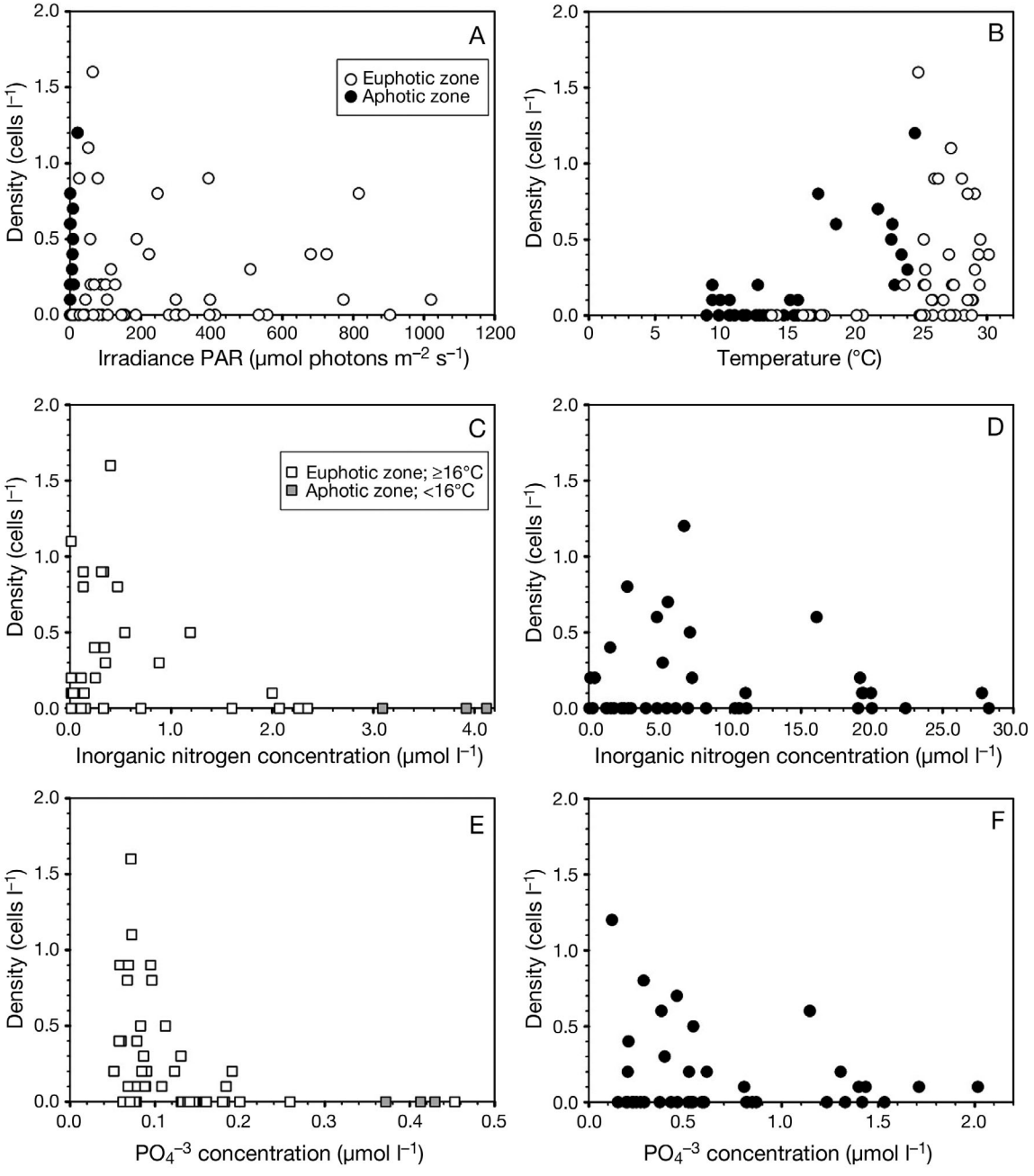

Fig. 6. Endosymbiont-bearing dinoflagellates. Relationship between cell density and (A) irradiance; (B) temperature; inorganic nitrogen concentration in the (C) euphotic zone and (D) aphotic zone; and PO_4^{3-} concentration in the (E) euphotic zone and (F) aphotic zone. PAR = photosynthetically active radiation

cell concentrations decreased as a function of temperature down to 10°C (Pearson's correlation coefficients, $p < 0.01$; Table 3, Fig. 6B). Some cells were, however, still present at temperatures <10°C. Nevertheless, a significant correlation was found between temperature and cell density (Table 3, Fig. 6B).

The highest cell concentrations of endosymbiont-bearing dinoflagellates in the euphotic zone were found when inorganic nitrogen concentrations were low (<1 µmol l^{-1}; Fig. 6C). Yet, the correlation between cell density and the concentration of inorganic nitrogen was not significant (Table 3). In the aphotic zone, the concentrations of inorganic nitrogen were considerably higher than in the euphotic zone (Fig. 6D). However, no correlation between cell concentration and inorganic nitrogen was found.

The endosymbiont-bearing dinoflagellates were most abundant in the euphotic zone at low PO_4^{3-} concentrations (Fig. 6E), and a negative correlation between cell densities and PO_4^{3-} concentrations was found ($p < 0.01$; Table 3). Such a negative correlation was not found in the aphotic zone (Fig. 6F, Table 3).

Light microscopy

All *Ornithocercus* spp. and *Histioneis* spp. cells examined contained extracellular cyanobacterial ectosymbionts, which were located within the cingulum (Fig. 7A–E). Many *Ornithocercus* spp. cells also had large rod-shaped non-photosynthetic bacteria on their

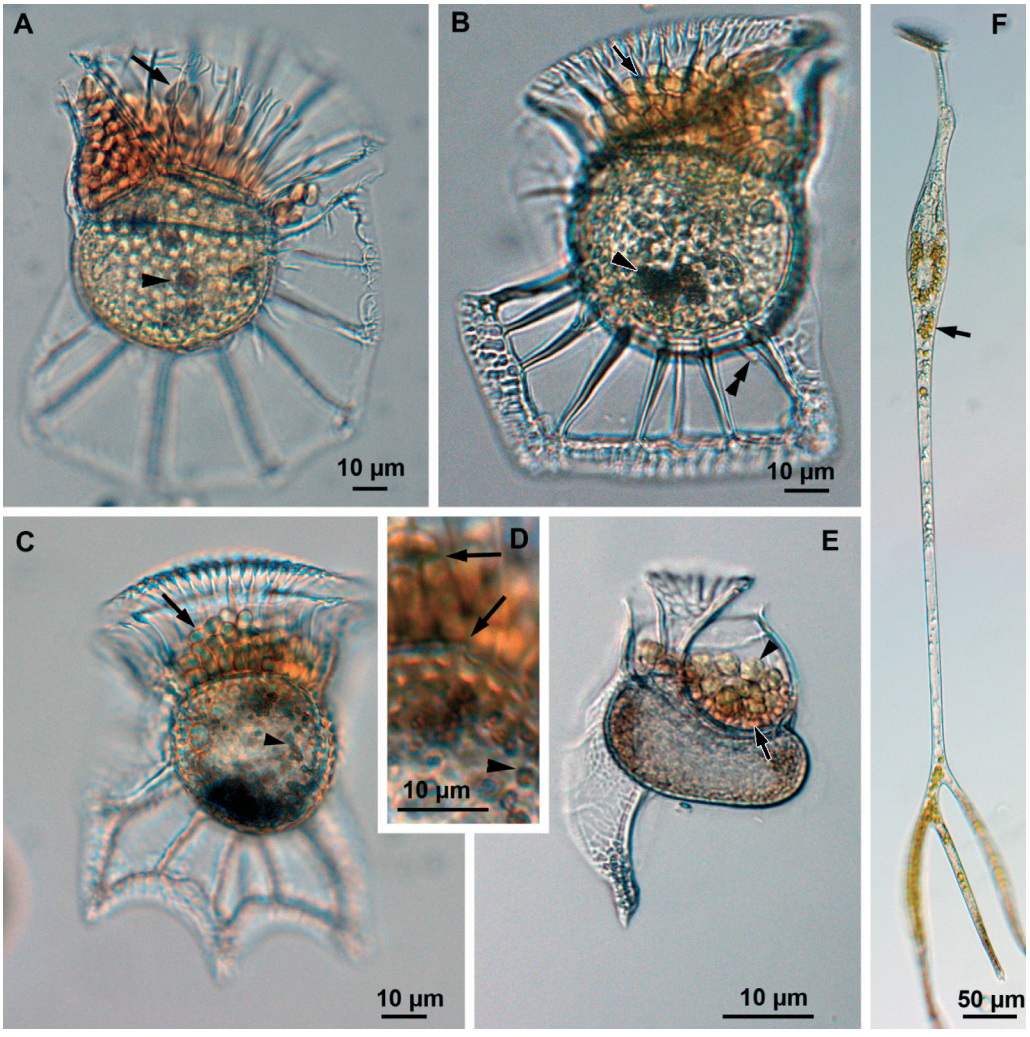

Fig. 7. Light microscopy of live cells. (A) *Ornithocercus steinii* with numerous cyanobacterial ectosymbionts present in the cingulum (arrow). Food vacuoles (arrowhead). (B) *O. quadratus*: cyanobacterial ectosymbionts (arrow), food vacuole (arrowhead), large bacteria (double arrowhead). (C) *O. magnificus*: cyanobacterial ectosymbionts (arrow); note the small putative food vacuoles with the same colour as the cyanobacterial ectosymbionts (arrowhead). (D) Detail of the same cell as in (C), with arrows showing the length of a cyanobacterial ectosymbiont. (E) *Histioneis carinata*: 2 different types of cyanobacterial ectosymbionts are present (arrowhead and arrow). (F) *Amphisolenia thrinax*: yellowish endosymbionts (arrow)

sulcal lists (Fig. 7B). Ectosymbiotic bacteria were not observed on cells of *Histioneis* spp. or *Amphisolenia* spp. Most cells of *Ornithocercus* spp. contained numerous food vacuoles of various sizes (Fig. 7A–D). In some cases they had a striking resemblance to the cyanobacterial ectosymbionts, having the same size and colour (Fig. 7A). In other cases inclusions were very small and in a state of degradation (Fig. 7C,D). In the genus *Amphisolenia*, only endosymbionts were found (Fig. 7F).

TEM

Cells of *Ornithocercus magnificus* and *O. quadratus* were examined using TEM. All cells had the typical dinoflagellate organelles including a nucleus with condensed chromosomes (dinokaryon) situated in the posterior part of the cell (Fig. 8A). In both species, cyanobacterial ectosymbionts were present in the cingulum (Fig. 8A), though a substantial number were lost during the fixation process. The cingulum also contained small rod-shaped heterotrophic bacteria (Fig. 8A).

An electron-dense layer, about 0.20 µm in thickness, subtended the thecal plates just below the inner plate membrane (Fig. 8A). It appeared to be a more or less continuous layer around the cell, though at some places it was interrupted and less dense. Here it became evident that the layer consisted of stacked membranes. An extensive number of rod-shaped bodies, so-called rhabdosomes, were scattered throughout the cytoplasm (Fig. 8A). They measured about 3 µm in length and 0.25 µm in width. Inside the rod or cylinder was a coiled electron-dense tube situated in a less dense matrix (Fig. 8B). The tube seemed to be attached to the putative posterior part of the cylinder and terminated in a funnel-like structure at the slightly pointed 'anterior' end (Fig. 8B). The rhabdosomes were never seen associated with the cell surface or the thecal pores.

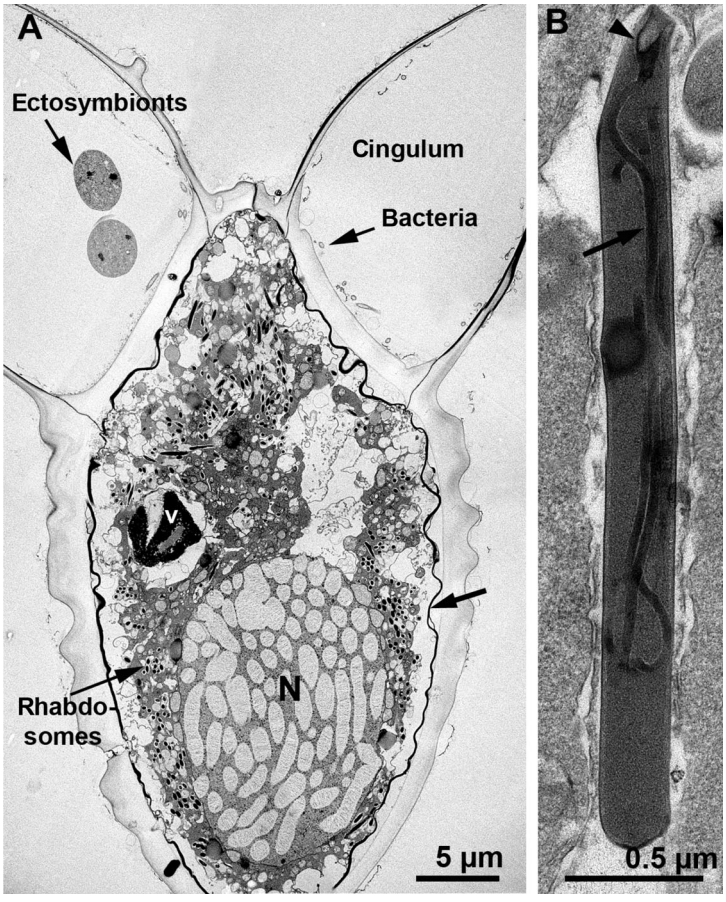

Fig. 8. Transmission electron microscopy of *Ornithocercus magnificus*. (A) Longitudinal section of a cell. Two cyanobacterial ectosymbionts and small bacteria are present in the cingulum. Numerous rhabdosomes are present within the cell. A dense membrane layer subtends the thecal plates (arrow). Nucleus (N), food vacuole (V). (B) Longitudinal section of a rhabdosome. The inner coiled tube (arrow) terminates in a funnel-like structure (arrowhead)

The *Ornithocercus* spp. cells contained a 'cytostome' surrounded by thin plates. A microtubular strand, similar to the peduncle microtubular strands observed in a number of other dinoflagellates, was located inside the cytostome (Fig. 9A). The cytostome was not studied in detail but appeared to be similar to that reported in *Dinophysis* spp. (Jacobson & Andersen 1994).

All cells sectioned (7 *Ornithocercus magnificus* and 2 *O. quadratus* cells) contained food vacuoles. The contents of these were usually in such a state of degradation that the identity of prey items was impossible. Most food vacuoles contained numerous rod-shaped trichocyst-like bodies with a diameter of about 500 nm, which most likely came from an ingested ciliate (Fig. 9B,C,F). One food vacuole contained remnants of a chloroplast having an internal rod-shaped pyrenoid and parallel-running thylakoid bands (Fig. 9E). The chloroplast was located close to a trichocyst-like body (Fig. 9D).

DISCUSSION

The role of symbionts for the dinoflagellates

Ectosymbionts

In the present study, light-microscopic observations revealed striking similarities between some of the food vacuoles and the extracellular symbionts, the same colour, size and shape was evident. However, in TEM sections, the

food vacuole contents were in such a state of degradation that we could not confirm this. Nevertheless, Lucas (1991) observed remnants inside a food vacuole resembling the extracellular symbionts in his TEM preparations, suggesting that the heterotrophic dinoflagellates may ingest the symbionts. In other words, it

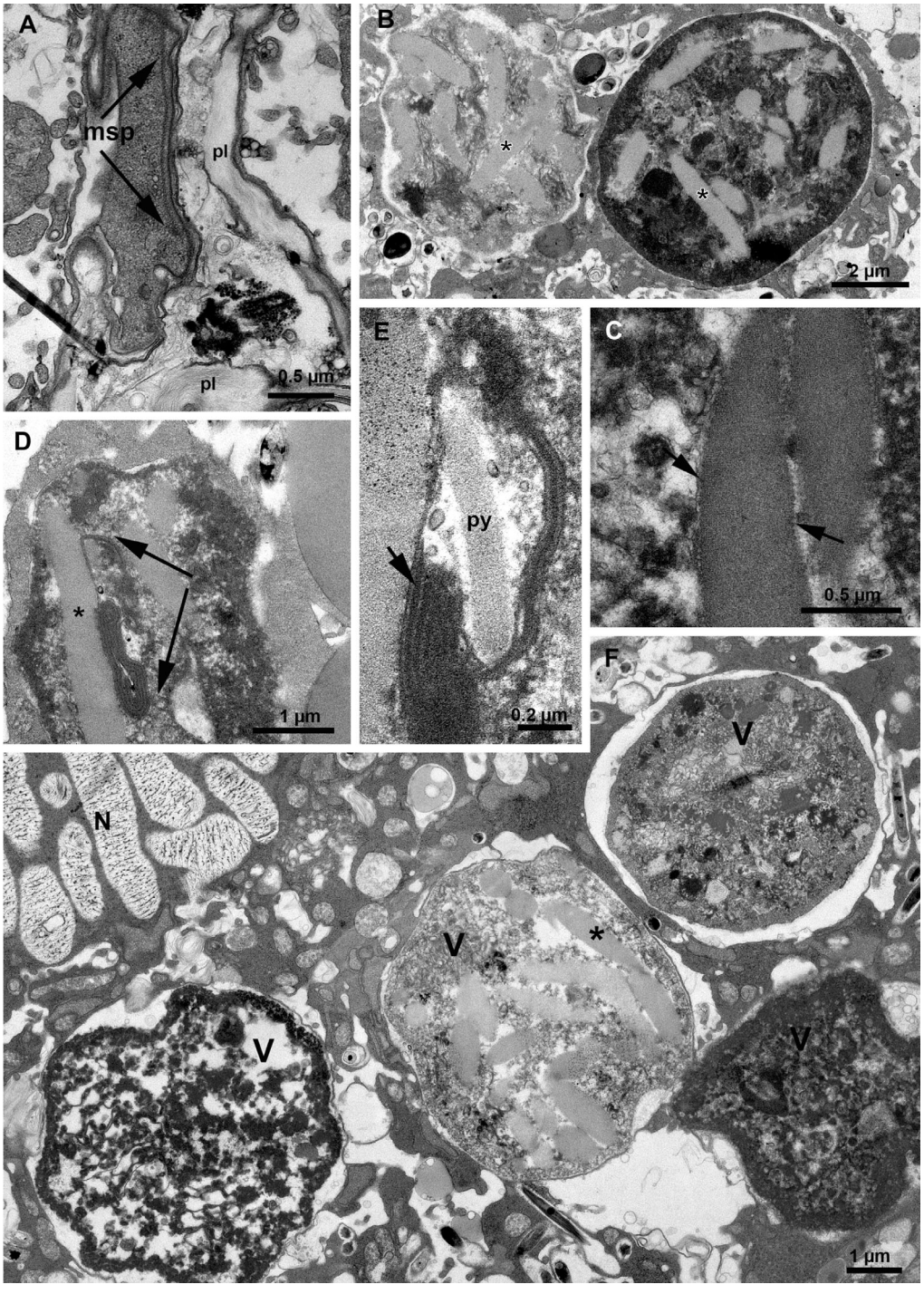

Fig. 9. Transmission electron microscopy of (A–E) *Ornithocercus magnificus* and (F) *O. quadratus*. (A) Inner part of the 'cytostome' delineated by thin thecal plates (pl). msp: microtubular strand of the peduncle. (B) Two 'typical' food vacuoles with numerous rod-shaped trichocyst-like structures (*; also in other panels). (C) Details of trichocyst-like structure within a food vacuole. Note the delicate substructure of the surface (arrows). (D) Food vacuole with remnants of a chloroplast (arrows). (E) Another section of the food vacuole in (D) revealing a pyrenoid (py) and thylakoids bands of the chloroplast (arrow). (F) Four different food vacuoles (V) with various partly digested prey remnants. N: nucleus

looks like the heterotrophic dinoflagellates are growing their own 'vegetables'.

However, the ectosymbiont-bearing dinoflagellates are probably not entirely dependent on cyanobacteria as a food source. It is well known that other members of the order Dinophysiales feed on ciliates by the use of a peduncle (e.g. *Dinophysis* spp. and *Oxyphysis* spp.; Elbrächter 1991, Hansen 1991, Park et al. 2006). In food vacuoles of *Ornithocercus* spp., we found trichocyst-like remnants that most likely originated from ciliates. The trichocyst-like structures we observed are of similar type to those found in food vacuoles of *Oxyphysis oxytoxoides* (Jacobson & Andersen 1994, their Figs. 44 & 45).

We also found remnants of a chloroplast in one of the food vacuoles of *Ornithocercus* spp. The identity of this chloroplast is uncertain, but its pyrenoid bears some resemblance to the pyrenoids found in certain prymnesiophytes, e.g. *Chrysochromulina apheles* (Moestrup & Thomsen 1986, their Fig. 8). Small prymnesiophytes did account for a substantial amount of the nanoplanktonic biomass in the samples taken (W. Tarangkoon et al. unpubl. obs.), but the close proximity of the chloroplast remnants to the trichocyst in the TEM section, and the scarcity of such degraded chloroplasts within food vacuoles in general in the *Ornithocerus* spp. cells indicated that algal cells were ingested by the ciliate before they entered *Ornithocercus* spp.

How the ectosymbiont-bearing dinoflagellates feed is unknown, but the presence of a cytostome with a microtubular strand similar to that found in *Dinophysis* spp. suggests that *Ornithocercus* spp. also uses a peduncle in food uptake (Hansen 1991, Jacobson & Andersen 1994). Also, Taylor (cited by Lucas 1991) observed a veil-like structure extruded from the flagellar pore of *Ornithocercus* sp.

We also found rhabdosomes in *Ornithocercus* spp., which have only been described in detail in *Dinophysis acuminata* (Vesk & Lucas 1986). Lucas & Vesk (1990) found a few rhabdosomes close to the apical pore of *D. acuminata* but no obvious signs of emission have ever been observed. Rhabdosomes are believed to function as trichocysts and be involved in prey capture (Lucas & Vesk 1990), but more research is required to understand their function.

Endosymbionts

Light-microscopic observations revealed numerous healthy endosymbionts inside *Amphisolenia* spp. Based on the present observations and what is known from the literature, these symbionts can either be eukaryotic or prokaryotic and thus may play different roles with regard to N_2 fixation (Lucas 1991). There is no doubt that the symbionts perform photosynthesis, but to what extent the heterotrophic dinoflagellates actually ingest the symbionts is unknown. It is, however, interesting that Lucas (1991) found dividing symbionts inside *A. globifera*, suggesting that the symbionts multiply inside the heterotrophic dinoflagellate. To what extent these symbionts are permanently incorporated into the heterotrophic dinoflagellates is presently unknown.

Spatial distribution of symbiont-bearing dinoflagellates

Surface temperatures ranged from 12 to 30°C during the cruise. The highest cell concentrations and species diversity were found at temperatures >20°C (Figs. 5B & 6B, Table 2). Dinoflagellates bearing either ecto- or endosymbionts in the photic zone were not observed at temperatures below 16 and 23°C, respectively. We found symbiont-bearing dinoflagellates at lower tem-

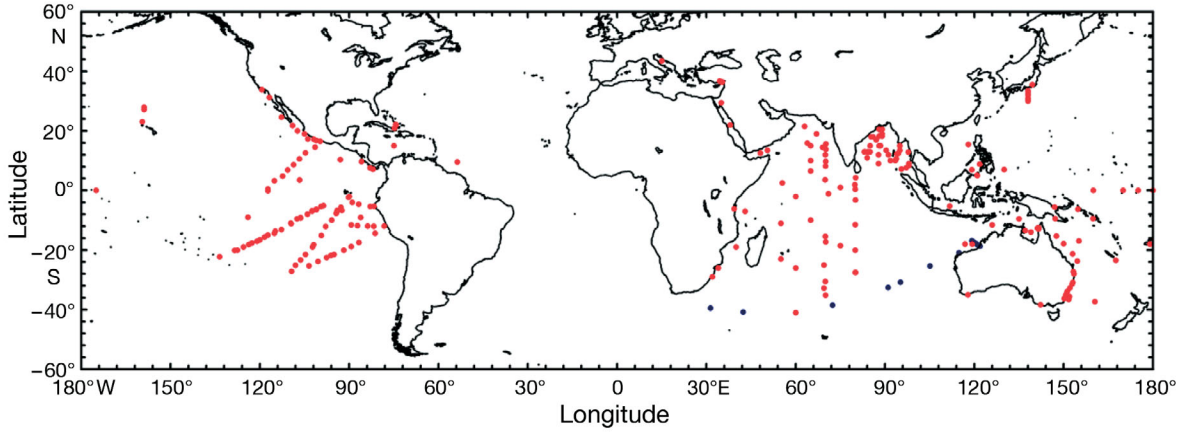

Fig. 10. Geographic distribution of dinoflagellates with symbionts. Data from references in Table 4 (red dots) and data from the present study (blue dots)

Table 4. Geographical distribution of symbiont-bearing dinoflagellates. Cit: *Citharistes*, His: *Histioneis*, Para: *Parahistioneis*, Orn: *Ornithocercus*, Amp: *Amphisolenia*, Tri: *Triposolenia.* na: data not available

Latitude	Longitude	Region	Surface temperature (°C)	Genera	Cell concentration (cells l^{-1})	Source
41–46° N	12–18° E	Adriatic Sea	10[a]–26	Orn, His, Amp, Tri	na	Vilićić et al. (2002)
36° 43′ N–36° 53′ N	35° 50′ E–33° 02′ E	Bay of İskenderun, Mediterranean Sea	15.8–28.9	Orn, Amp	na	Polat et al. (2000)
36° 23′ N–36° 33′ N	35° 22′ E–35° 23′ E	Turkish coast (Karatafl–Adana), Mediterranean Sea	15.5–29	Orn	na	Polat & Iflik (2002)
36° 06′ N–36° 07′ N	33° 28′ E–33° 30′ E	Turkish coast, Mediterranean Sea	16.6	His	na	Polat & Koray (2002)
36° 07′ N–36° 54′ N	33° 24′ E–36° 11′ E	Northern Levantine basin, Mediterranean	15–16, 29–30	Cit, Orn, His, Amp	na	Polat & Koray (2007)
~35–36° N[b]	139–140° E[b]	Coast of Japan, Pacific Ocean	na	Cit[c], Orn, His, Para[c], Amp, Tri	na	Abé (1967)
na	na	Tropical and subtropical Atlantic	na	Cit, Orn, His, Amp	na	Foster et al. (2006b)
na	na	North Atlantic Ocean	na	Cit, Orn, His, Para, Amp	na	Lessard & Swift (1986)
29° 30′ N	~35° E	Gulf of Aqaba, Red Sea	na	His, Para	2.5–3	Kimor et al. (1992)
29° 30′ N	~35° E	Gulf of Aqaba, Red Sea	21–26	Cit, Orn, His	2.5–3.5	Gordon et al. (1994)
~33° N–30° S	~79–133° W	Pacific Ocean	18.3–29.4	Cit, Orn, His, Para, Amp, Tri	na	Kofoid (1907), Kofoid & Michener (1911), Kofoid & Skogsberg (1928)
23° 44.91′ N, 27° 58.61′ N	159° 27.80′ W, 158° 53.86′ W	Northwest of Hawaii, Pacific Ocean	na	Orn, His	na	Foster et al. (2006a)
20° 54′ N–22° 10′ N	73° 48′ W–74° 21′ W	Bahamian waters, North Atlantic Ocean	28.8–29.1	Cit, Orn	na	Carpenter et al. (1991)
12° 17′ 21″ N	93° 59′ 06″ E	Barren Island region, Andaman Sea	29.1–29.5	Orn	1	Eashwar et al. (2001)
~10–20° N[b]	~60–90° W[b]	Caribbean Sea, Atlantic Ocean	na	Orn	na	Janson et al. (1995)
11–20.5° N	80–89° E	Bay of Bengal	28–30.5	Orn, His	0.4–5.0	Jyothibabu et al. (2006)
09° 34.78′ N	53° 39.45′ W	Northeast of Brazil, North Atlantic Ocean	na	Orn	na	Foster et al. (2006a)
09–23° N	56–69° E	Arabian Sea	na	Orn	na	Dennett et al. (1999)
7–22° N	80–98° E	Bay of Bengal	na	Cit, Orn, His, Para, Amp, Tri	na	Taylor (1976)
7–20° N	81–88° E	Bay of Bengal	27–28	Amp	na	Paul et al. (2007)
7–14° N	90–95° E	Bay of Bengal and Andaman Sea	27–29	Orn, Amp	na	Jyothibabu et al. (2003)
0° 23′ N–16° 30′ N	79° 58′ E–85° 32′ E	Indian Ocean	22.94–30.52	Orn, His, Para		Norris (1967)
~0° N–33° 30′ N	~118° E–175° W	Western Pacific Ocean	22–29.5	His	5.0, 32[d]	Gómez (2005)
na	na	Equatorial Pacific Ocean	na	Cit, Orn, His, Amp	na	Foster et al. (2006b)
01° 07′ S–35° 09′ S	71° 00′ E–69° 59′ E	Indian Ocean	15.03–28.95	Orn, His, Amp	na	Norris (1967)
~10° S–28° S	~112–155° E	Australian region	~25–30	Cit, Orn, His, Para, Amp	na	Hallegraeff & Jeffrey (1984)
~10° S–40° S[b]	~110–160°E[b]	Australian region	na	Cit, Orn, His, Para, Amp	na	Wood (1954, 1963a,b,c)
13° 06′ S–13° 30′ S	141°06′ E–141°48′ E	Gulf of Carpentaria, Australia	25–32	Orn, His, Amp	na	Burford et al. (1995)
34–37° S	150–153° E	East coast of Australia	20–21	Orn, His, Amp	na	Jeffrey & Hallegraeff (1987)
39°S–19°N	32–80° E	Indian Ocean	na	Orn, His, Para, Amp, Tri	na	Taylor (1976)

[a]Lowest temperature in the region investigated, but it was not reported whether any dinoflagellates bearing symbionts were found at this temperature
[b]We estimated the coordinates according to the region as named in the literature
[c]Precise location unknown
[d]Only 1 sample with this concentration

peratures, but this was always in the aphotic zone and it cannot be ruled out that these cells represent cells sinking from the euphotic zone.

A literature survey on the geographical distribution of symbiont-bearing dinoflagellates (i.e. the genera *Amphisolenia*, *Citharistes*, *Histioneis*, *Ornithocercus*, *Parahistioneis* and *Triposolenia*) in combination with the present data set suggest that their distribution is restricted to tropical, subtropical and warm-temperate seas from about 46° N to 40° S (summarised in Table 4, Fig. 10). Previous reports have focused on species diversity (not cell density) in an area with often very little information on temperature and exact location of samples containing symbiont-bearing dinoflagellates. Thus, reported surface temperatures are therefore often only given as ranges within geographical areas visited or as seasonal ranges. Nevertheless, symbiont-bearing dinoflagellates have only been reported with certainty in waters which have a surface temperature in the range of roughly 15 to 30°C (Table 4). This is in accordance with our results.

The reasons for the apparent dependency on fairly high temperatures in these organisms are unknown, as none of them yet have been cultured in the laboratory. It may be a direct temperature effect on the heterotrophic dinoflagellate or its symbionts. However, a number of indirect factors may also lead to this apparent high temperature requirement, including a lack of seasonality in light and inorganic nitrogen concentration.

In the present study, symbiont-bearing dinoflagellates were mainly found in the euphotic zone, suggesting that they rely on light for photosynthesis of their symbionts. Cells were also found below the euphotic zone, as far down as 200 m and on some occasions 400 m, but generally in much lower cell densities (Fig. 4). Similar vertical distributions of these dinoflagellates have been found previously in other parts of the world (Kofoid & Skogsberg 1928, Gordon et al. 1994, Gómez 2005, Jyothibabu et al. 2006).

It is evident from our data set that the highest cell concentrations of symbiont-bearing dinoflagellates were found in warm waters characterised by very low nutrient and chl *a* concentrations (Figs. 3B,D, 5 & 6, Table 3). However, it is important to note that these dinoflagellates were not very abundant in the Broome coastal waters with water depths below 150 to 200 m, even though inorganic nitrogen and phosphorous concentrations were very low (Figs. 2 & 3). So, although nutrient concentration and temperature are important factors determining the distribution of these organisms, other factors must play an important role as well.

In coastal waters, transport of nutrients from land or from mixing of the water column will increase primary production compared to offshore waters, allowing increased zooplankton biomass and thereby increased turnover rate of production. A. W. Visser et al. (unpubl.) documented considerable water mixing at the first 3 stations along the Indian Ocean transect transporting nutrients from the pycnocline to the surface waters, leading to increased primary productions at these stations. Also, data published from the Indian Ocean transect (same cruise) show that the copepod biomass at the coastal stations on the Indian Ocean transect was 4-fold that of the more oceanic stations (Jaspers et al. 2009). Thus, turnover rates in the planktonic food web are definitely higher in the coastal waters of the Indian Ocean transect. Symbiont-bearing dinoflagellates are in general quite large cells and most likely have fairly low maximum growth rates (Hansen et al. 1997) Thus, in coastal waters where turnover rates of organic matter are high, these dinoflagellates will probably not be able to outgrow the losses due to grazing.

CONCLUSIONS

High abundances and species diversity of symbiont-bearing dinoflagellates were found in the photic zone in warm offshore waters characterised by low nutrient and chl *a* concentrations. We found indications that ectosymbiont-bearing dinoflagellates not only seem to ingest their ectosymbionts, but also ingest other prey items such as ciliates. Thus, it seems that these dinoflagellates cope with low-nutrient environments by using a multi-resource strategy. To what extent this also applies to endosymbiont-bearing dinoflagellates is unknown at present. Rates of growth, photosynthesis and N_2 fixation as well as grazing loss rates of symbiont-bearing dinoflagellates are still lacking in the literature. More research is needed to unravel their functional biology and understand their role in the pelagic food web.

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