

C. P. Marquis · A. H. Baird · R. de Nys
C. Holmström · N. Koziumi

An evaluation of the antimicrobial properties of the eggs of 11 species of scleractinian corals

Received: 28 February 2004 / Accepted: 29 November 2004 / Published online: 19 May 2005
© Springer-Verlag 2005

Abstract Potential sources of mortality of marine invertebrate larvae are numerous and include predation and diseases caused by marine microorganisms. Extracts from the eggs of 11 coral species were evaluated for their ability to deter surface attachment and inhibit the growth of two marine tolerant laboratory bacteria and 92 bacterial strains isolated from seawater and the surface of coral colonies on the Great Barrier Reef (GBR). Extracts of the eggs of *Montipora digitata* inhibited the growth of the two laboratory bacteria, *Vibrio harveyi* and *Bacillus subtilis*, and one bacterial isolate from the mucus of the coral *Favia pallida* in disc diffusion and liquid culture assays. No other microbial strains ($n=91$) from the surface of corals and the reef environment were inhibited by *M. digitata* extracts. No antibacterial activity was found in the egg extracts of the remaining ten coral species and none of the extracts inhibited surface attachment of various bacteria. Extrapolation of estimated surface concentrations of the biologically active extract of *M. digitata* suggests that the level of the growth inhibitory compounds may be sufficient to deter microbial growth *in situ*.

Keywords Bioactivity · Antimicrobial · Antifouling · Chemical ecology · Coral reef

Introduction

Reproduction of scleractinian corals on the Great Barrier Reef and in other parts of the world, is highly synchronized. On the GBR, the majority of coral species spawn predictably during 1 week in late spring (Harrison and Wallace 1990) and over 30 species may spawn on the same night on a single reef (Willis et al. 1985). Depending on the prevailing weather conditions, larvae aggregate into large slicks on the surface of the ocean that persist for 4–48 h. With such a high density of larvae, and the nutritional potential of such a “medium” to facilitate bacterial growth and colonization, some form of antibiotic defense might be desirable. Indeed, in culture, coral larvae appear remarkably resistant to contamination, and the aseptic techniques that are necessary in the aquaculture of many other invertebrate larvae are unnecessary when culturing corals (Babcock et al. 2003).

Many marine taxa produce specific compounds to defend themselves against the settlement and growth of microorganisms. For example, extracts of various developmental stages of the soft coral, *Parerythropodium fulvum fulvum*, are toxic to potentially pathogenic bacteria including a *Vibrio* sp. (Kelman et al. 1998). Inhibition of surface attachment is another possible mechanism by which bacterial colonization and pathogenesis may be deterred (Maximilien et al. 1998; Steinberg et al. 2001, 2002). For example, extracts of Antarctic soft corals exhibit antifouling activities to sympatric strains of bacteria (Slattery et al. 1995) and the subtidal red algae *Delisea pulchra* produces a range of halogenated furanones with strong antifouling activity (de Nys et al. 1995) and deterrence of bacterial colonization (Givskov et al. 1996; Maximilien et al. 1998). Chemical defense has also been demonstrated in many marine larvae (Lindquist 2002), though studies are largely restricted to compounds that deter larval predation.

Communicated by Biological Editor H.R. Lasker

C. P. Marquis (✉) · C. Holmström · N. Koziumi
School of Biotechnology and Biomolecular Sciences,
University of NSW, Sydney, NSW, 2052, Australia
E-mail: c.marquis@unsw.edu.au

A. H. Baird
Centre for Coral Reef Biodiversity,
School of Marine Biology and Aquaculture,
James Cook University, Townsville, QLD, 4811, Australia

R. de Nys
School of Marine Biology and Aquaculture,
James Cook University, Townsville, QLD, 4811, Australia

R. de Nys · C. Holmström
Centre for Marine Biofouling & Bioinnovation,
University of NSW, Sydney, NSW, 2052, Australia

Under conditions of environmental stress, such as pollution (Segal and Ducklow 1982; Mitchell and Chet 1975) or elevated temperature (Kushmaro et al. 1996), bacteria may become pathogenic to corals, initiating disease such as Black Band disease (Antonius 1985), causing tissue necrosis (Hodgson 1990), and loss of coral symbionts i.e. bleaching. However, studies on the extracts from a number scleractinian species suggest these organisms produce biologically active substances that can adversely affect potential pathogens, competitors, and conspecifics (Gunthorpe and Cameron 1990a–c; Fearon and Cameron 1996). Koh (1997) found activity against a range of both marine and terrestrial bacteria with significant differences in activity between families, with extracts from the Dendrophylliidae being most active. Gunthorpe and Cameron (1990a, b) found variable intra-specific activity, as well as species, seasonal, and spatial differences in the antimicrobial activity of extracts. Antimicrobial activity of the extracts was negatively associated with the presence of immature gonads, which suggested that reproductive status was a significant predictor of antibiotic activity (Gunthorpe and Cameron 1990a). A similar phenomenon was reported where diterpenes were selectively incorporated into egg-lipid material of the octocoral *Lobophytum compactum* (Bowden et al. 1985). Eggs contained the diterpene thunbergol, which was barely detectable in adults before spawning and absent afterwards (Bowden et al. 1985).

Studies on the chemical ecology of the eggs of Scleractinia have been limited. The damselfish *Pomacentrus mollucensis* clearly preferred the eggs of two acroporid species over the eggs from *Pachyseris speciosa* (Pratchett et al. 2002) and assays of the extracts of these eggs suggested that they are chemically defended (Baird et al. 2001). In addition, extracts of the eggs of one coral species, *Montipora digitata*, possess chemicals which inhibit the growth of the bacterium *Escherichia coli* (Fusetani et al. 1996). Further studies have indicated that these chemicals exhibit anti-tumour activity (Bae et al. 2000; Alam et al. 2001). Bioactivity in coral tissues has been reported by a number of investigators, however, studies of the antimicrobial properties of coral eggs are limited to a single species. This research examined the antimicrobial activity of extracts from the eggs of 11 coral species of the GBR against two laboratory strains of marine bacteria and ninety two microbial isolates from the GBR. The ability for extracts to deter microbial surface attachment was also evaluated.

Methods

Collection of coral eggs

All collections were undertaken on the reefs around Orpheus and Pelorus Island (18°46'S, 146°15'E), QLD, Australia in December 1999. Four to six gravid colonies of each of the coral species were collected and transported to separate culture tanks (one species per tank) at

Orpheus Island Research Station (OIRS). Following spawning, eggs from each tank were collected using 200 µm sieves (see also Babcock et al. 2003) washed in sterile seawater and frozen at –20°C. Frozen eggs were then transported to the University of NSW for extraction. Eggs from the following coral species were collected: *Acropora elseyi*, *A. tenuis*, *A. hyacinthus*, *A. valida*, *A. cerealis*, *A. millepora*, *Echinopora lamellina*, *Goniastrea favulus*, *Favia pallida*, *Lobophyllia pachysepta* and *M. digitata*.

Extraction of coral eggs

Frozen eggs were freeze-dried and the dried materials were then extracted with methanol and dichloromethane (1:1) and then with absolute ethanol. DCM/methanol solvent was used to extract lipid-soluble fractions whereas ethanol was used to extract more polar components. Approximately three times the volume of solvent in relation to the volume of dried egg was added for each extraction. After 12–24 h extraction, the solvent was transferred into centrifuge tubes and then centrifuged (1,500 g for 3 min) to pellet any fine residue. This procedure was repeated twice with DCM/methanol (1:1). The percentage solubility of the lyophilized eggs in DCM:Methanol was determined gravimetrically from the mass of the dried residue after extraction and comparing to the original dry weight prior to extraction. The remaining solids were then extracted with absolute alcohol using a similar procedure. The pooled DCM/methanol and ethanol extracts were then air-dried. Twenty-one extracts were obtained for bioactivity studies (11 DCM/methanol extracts, one from each species plus ten ethanol extracts, one from each species except *Goniastrea favulus*, where insufficient material was available for the ethanol extraction).

Microbiology

To investigate the susceptibility of native strains of bacteria to extracts from coral eggs, bacteria were isolated from the coral reef environment. Water samples were collected using sterile syringes from the water column and from the surface of coral colonies. Samples were transported to the laboratory and plated out at various dilutions on to VNSS agar plates (Holmström et al. 1998). Ninety-two bacterial isolates were obtained using traditional microbiological methods. These isolates were characterized by colony morphology, pigmentation and reaction to 3% KOH to establish Gram status (Gregersen 1978).

Laboratory strains of common marine bacteria *Bacillus subtilis* (strain number 0003000, University of NSW Culture Collection) and *Vibrio harveyii* (strain number 47–6661, Australian Collection of Marine Organisms, James Cook University) were selected as examples of marine-tolerant Gram positive and Gram

negative bacteria. In addition, members of the genus *Vibrio* have been implicated in a number of diseases of marine organisms.

Disc diffusion assay

Disc diffusion assays were used to test whether egg extracts inhibited the growth of selected bacteria (Russell and Quesnel 1983). The dried extracted material was first dissolved in the original solvent (either DCM/methanol (1:1) or ethanol (25 mg/ml). Twenty microlitres of extract solution was pipetted onto paper discs (Oxoid, UK) resulting in the application of 500 µg per disc (Koh 1997; Gunthorpe and Cameron 1990a). Discs were air-dried for 48 h prior to use in the disc diffusion assay. Based on the surface area of the 6 mm discs, this treatment resulted in an estimated surface concentration of extract of 1,000 µg cm⁻² on each disc. Positive controls for bacterial growth inhibition were prepared using solutions of the antibiotic gentamicin (25 mg/ml). Untreated and solvent-only discs were used as negative controls. Duplicate spread plate cultures of the microorganisms were prepared on VNSS agar plates and the discs placed on the agar plates. The agar plates were incubated overnight at 30°C and examined for zones of inhibition the following day.

Liquid culture growth inhibition assay

Growth inhibition studies were also performed in liquid culture to examine the direct effects of extracts on bacterial growth rates. Disc diffusion assays rely on diffusivity of bioactive compounds for a zone of inhibition to form. The liquid culture assay was performed to overcome potential diffusional limitations. Twelve bacterial strains isolated from reefs around Orpheus and Pelorus Island and two laboratory strains, *B. subtilis* and *V. harveyi*, were used. These 12 isolates were selected from the original 92 to encompass a range of colony morphology, pigmentation, and site of isolation. Twenty-one coral egg extracts were tested against these 12 bacterial isolates. Bacteria, in early log-phase growth, were added into test tubes to a final volume of 10 ml and paper discs with extracts as prepared above, were placed into the culture, giving a maximum total extract concentration of 50 µg/ml. The cultures were incubated at 30°C with agitation. The bacterial cultures were sampled every hour and the bacterial cell density was estimated by measurement of the optical density at 650 nm using a plate reader (Emax, Molecular Devices, USA).

Analysis of liquid culture growth experiments

Growth rates were not readily described by typical exponential growth models due to likely oxygen limita-

tion resulting from the culture technique employed. During the growth period, the rate of increase in cell density was best approximated by a linear model, with a constant rate of biomass change over time assumed, which is a pattern typical of oxygen-limited culture of aerobic organisms. Consequently, the growth rates of bacteria populations in the presence and absence of coral egg extracts, were estimated using linear regression. Growth rates estimated using this method were compared between controls and test solutions. The slopes of the lines of test samples were compared to the untreated control using an ANCOVA (analysis of covariance) method to a 99% confidence interval (Zar 1999).

Bacterial adhesion assay using the Laser Scanning Cytometer (LSC)

To test whether coral egg extracts inhibited the adhesion of microbes to a surface placed in a marine environment, glass slides were coated with extracts. Glass slides were cleaned and autoclave tape was used to mark out a 1 cm² area on each slide. The dried extracted material was dissolved in the original solvent (either DCM/methanol (1:1) or ethanol at a concentration of 25 mg/ml and 20 µl of extract solution was pipetted onto glass slides in the 1 cm² area to give a surface concentration of 500 µg/cm². The slides were then placed on a rocking device to spread the extract evenly across the 1 cm² area whilst the solvent evaporated. Slides were coated with negative controls (DCM/methanol and ethanol solvent only), a positive control (furanone 2, a deterrent of bacterial attachment and growth (De Nys et al. 1995; Giskov et al. 1996) and 21 coral egg extracts. Each treatment was prepared in triplicate. Slides were placed into a large container filled with fresh seawater (obtained from Maroubra Beach, Sydney) that was gently agitated at room temperature (approximately 24°C). After 3 h the slides were removed and washed with filter-sterilized seawater to remove unattached bacterial cells. The attached bacteria were stained by immersion in an Acridine Orange solution (1 mg/ml in 2.5% glutaraldehyde) for 10 min, rinsed in sterile seawater and air-dried. Attached bacteria were enumerated using a Laser Scanning Cytometer (Compucyte Corp., USA) at an excitation wavelength of 500 nm and emitted light recorded at 530 nm. Three fields of view were counted and the mean value and standard deviation were determined for each slide. A one-way ANOVA was used to test for significant differences in the mean number of attached bacteria among the different treatments. The raw data were used following examination for normality of data using plots of residuals and Levene's test for homogeneity of variances. Tukey's Least Significant Difference test was used to determine treatments responsible for the differences detected.

Results

Extraction and disc diffusion assay

Ninety-two bacterial isolates (78 Gram negative, 14 Gram positive) were obtained from primary isolation plates of the 11 corals and the surrounding water column. Strains were differentiated on the basis of Gram status, colony morphology, and pigmentation. The laboratory isolates, *V. harveyi* and *B. subtilis*, plus the 92 isolates were assayed for susceptibility to each of the coral egg extracts. Freeze dried eggs were highly soluble in DCM:Methanol (65.0% w/w, SD 5.7%, $n=11$). Growth of the bacterial strains, *V. harveyi* and *B. subtilis*, were inhibited by DCM/methanol and ethanol extracts of *M. digitata* (Fig. 1 shows results for *B. subtilis*). Growth inhibition zones varied from 5–7 mm in diameter. None of the GBR bacterial isolates (i.e. resident isolates) were susceptible to either of the *M. digitata* extracts. No evidence of growth inhibition was found in any of the extracts from the other ten coral species.

Liquid culture assay

The growth-inhibitory properties of all extracts were assessed against 12 isolates from the GBR and the two

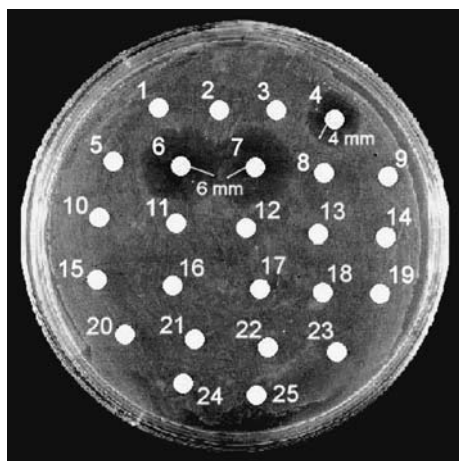


Fig. 1 Disc-diffusion assay for extract from coral eggs against *Bacillus subtilis*. 1 no coating, 2 DCM/methanol (1:1), 3 ethanol, 4 gentamycin, 5 furanone 2, 6 *Montipora digitata* DCM/methanol (1:1) extract, 7 *M. digitata* ethanol extract, 8 *Acropora elseyi* DCM/methanol (1:1) extract, 9 *A. elseyi* ethanol extract, 10 *A. hyacinthus* DCM/methanol (1:1) extract, 11 *A. hyacinthus* ethanol extract, 12 *A. millepora* DCM/methanol (1:1) extract, 13 *A. millepora* ethanol extract, 14 *A. valida* DCM/methanol (1:1) extract, 15 *A. valida* ethanol extract, 16 *A. cerealis* DCM/methanol (1:1) extract, 17 *A. cerealis* ethanol extract, 18 *Echinopora lamellosa* DCM/methanol (1:1) extract, 19 *E. lamellosa* ethanol extract, 20 *Lobophyllia pachysepta* DCM/methanol (1:1) extract, 21 *L. pachysepta* ethanol extract, 22 *A. tenuis* DCM/methanol (1:1) extract, 23 *A. tenuis* ethanol extract, 24 *Favia pallida* DCM/methanol (1:1) extract, 25 *Goniastrea favulus* DCM/methanol (1:1) extract

marine-tolerant laboratory strains. Consistent with results obtained in the disc diffusion assay, a significant reduction of bacterial growth (ANCOVA, $f_{1,6} \geq 129$, $P < 0.01$) occurred in DCM/methanol and ethanol extracts of *M. digitata* eggs against *V. harveyi* and *B. subtilis* (Fig. 2). In addition, the growth of one isolate (#5-3, a Gram +, rod-shaped bacteria) was significantly inhibited in liquid culture (Fig. 2). No further activity was apparent in the extracts from the remaining ten coral species.

Inhibition of surface attachment by coral egg extracts

None of the 21 coral extracts inhibited the attachment of bacteria to the panels immersed in seawater. For example, there was no significant difference in the mean bacterial cell counts per field of view among panels treated with ethanol extracts of *M. digitata* ($1,850 \pm 120$ SEM), DCM/methanol extract of *M. digitata* ($1,680 \pm 130$), and the untreated panel ($1,770 \pm 160$). Only the treatment with furanone significantly reduced the cell counts (746 ± 18) when compared to the untreated panel mean as determined by Tukey's HSD post hoc comparisons ($p = 0.034$).

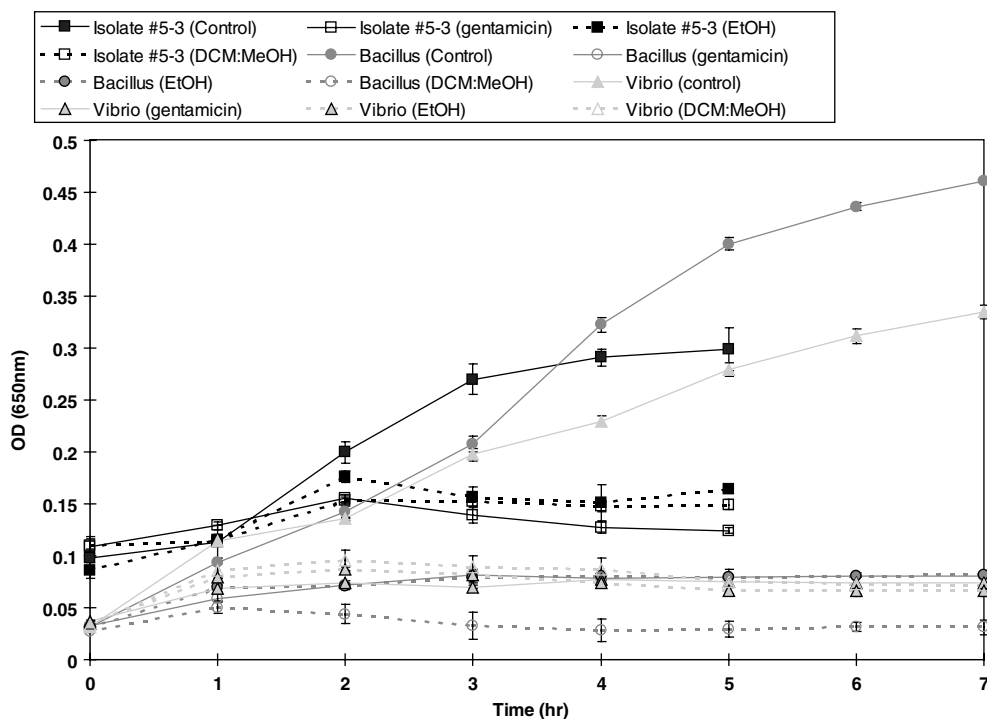
Discussion

Of the 11 coral species examined, only *M. digitata* showed evidence of antimicrobial activity. Similar antimicrobial activity of extracts of the eggs of *M. digitata* has been demonstrated against *E. coli* (Fusetani et al. 1996), with activity being attributed to a range of diacetylenic compounds (Alam et al. 2001). In this study, growth-inhibition was demonstrated against *B. subtilis*, *V. harveyi*, and a single isolate in the liquid culture growth assay. Resident strains were largely unaffected by the extracts.

Koh (1997) reported limited antimicrobial activity of coral tissue extracts against marine bacterial strains obtained from culture collections (2–5% of tissue extracts were active) but substantially higher activity against a terrestrial bacteria (*Staphylococcus aureus*, 27%) and a marine cyanobacterium (100%). Relatively specific antibacterial activity was demonstrated in aqueous and ethanol extracts, as compared to those obtained from corals of the Family Mussidae which most commonly demonstrates broad antibacterial activity (Gunthorpe and Cameron 1990a). In addition, *Gonipora tenuidens* secreted allochemicals that were toxic to and deterred the settlement of potential competitors, providing further evidence for active chemical defences in some scleractinian species (Gunthorpe and Cameron 1990c; Fearon and Cameron 1996).

The extracts of *M. digitata* eggs inhibited the growth of exotic bacteria but not local isolates. The resistance of sympatric microorganisms to extracts of the soft coral, *P. fulvum fulvum* has been previously

Fig. 2 Growth inhibition of *Vibrio harveyi* (triangles), *Bacillus subtilis* (circles) and isolate #5-3 (squares) by extracts of *Montipora digitata* in the liquid culture assay. Solid lines represent untreated and gentamicin controls. Dashed lines represent discs treated with egg extracts. Data points are mean values of three determinations of OD and the error bars represent the standard error of the mean



observed, whilst a potentially pathogenic *Vibrio* strain was growth-inhibited by crude extracts of the coral tissue (Kelman et al. 1998). The specific activity demonstrated at environmental concentrations suggest a role for these compounds in maintaining a natural microbial community. Similarly, the activity of the *M. digitata* egg extracts appears to be very specific in its action and also, interestingly, acts against an exotic *Vibrio* species. Bacteria from this genus have been implicated in causing disease in *P. fulvum fulvum* (Kelman et al. 1998), Caribbean gorgonians (Jensen et al. 1996; Harvell et al. 1996) and in coral bleaching (Kushmaro et al. 2001).

Evaluating the ecological significance of the antimicrobial activity in the *M. digitata* extracts relies on a valid comparison between the apparent concentration at the egg surface, with either the “surface concentration” of the compounds in treated discs (disc-diffusion assay) or the solution concentration in the liquid culture assay. Assuming a mean egg diameter of $337 \pm 6 \mu\text{m}$ for *M. digitata* (Baird et al. 2001) and an egg density of 0.99 g cm^{-3} (Arai et al. 1993), a relative surface concentration of the extractable fraction can be estimated, assuming that the bioactive compounds are evenly distributed throughout the egg material. Considering the mean DCM/Methanol fraction (65% of egg dry weight) results in a calculated surface concentration of $3,600 \mu\text{g.cm}^{-2}$ in the eggs of *M. digitata* compared with an estimated surface concentration of $1,000 \mu\text{g.cm}^{-2}$ in the treated discs. This suggests that the surface concentration of the growth inhibitory fraction was somewhat higher in the eggs

than on the test discs, indicating that observations of bacterial inhibition in our experiments should apply to egg surfaces. In the liquid culture experiments, the maximum total extract concentration in the solution equates to $50 \mu\text{g/ml}$. Without data on the mass fraction of bioactives in this extract and the solubility of the extract in the growth media, it is not possible to evaluate an actual minimum inhibitory concentration (MIC) for *M. digitata* extracts. However, growth inhibition equivalent to $50 \mu\text{g/ml}$ gentamicin was demonstrated in this assay for the laboratory strains, *V. harveyi* and *B. subtilis*, and the one susceptible marine isolate.

Bioactive compounds in found in marine organisms may be maternal in origin, provided by epibiotic microbiota (Holmström et al. 1998, 2002; Holmström and Kjelleberg 1999; Gil-Turnes and Fenical 1992) or potentially produced by endosymbiotic zooxanthellae (Ciereszko 1989). The origin of the antimicrobial compounds found in the eggs of *M. digitata* requires further investigation but interestingly, it is the only species in this study that incorporates zooxanthellae into the eggs prior to release.

Growth inhibition of selected exotic bacteria by extracts of the eggs of *M. digitata* has been confirmed in this study. The ecological significance of this finding remains uncertain. This property does not appear to be shared amongst other coral species. The presence of zooxanthellae in the eggs *M. digitata* suggests a possible role of the zooxanthellae in the production of these compounds but further experimentation is required to evaluate their potential role.

Acknowledgements This project was funded by an ARC Small Grant (UNSW) to CPM, R de N and AHB and a Project Aware Foundation Grant to CPM. We thank the staff at Orpheus Island Research Station for their assistance and support. M and D Pratchett provided invaluable assistance in the field. This is contribution number 270 of the Coral Ecology Group at James Cook University and 117 of the Centre for Coral Reef Biodiversity.

References

- Alam N, Bae BH, Hong JK, Lee CO, Im KS, Jung JH (2001) Cytotoxic diacetylenes from the stony coral *Montipora* species. *J Nat Prod* 64:1059–1063
- Antonius A (1985) Coral diseases in the Indo-Pacific: a first record. *Mar Ecol* 6(3):197–218
- Arai T, Kato M, Heyward A, Ikeda Y, Iizuka T, Maruyama T (1993) Lipid composition of positively buoyant eggs of reef building corals. *Coral Reefs* 12:71–75
- Babcock RC, Baird AH, Piromvaragorn S, Thomson DP, Willis BL (2003) Identification of scleractinian coral recruits from Indo-Pacific reefs. *Zool Stud* 42:211–226
- Bae BH, Im KS, Choi WC, Hong JK, Lee CO, Choi JS, Son BW, Song JI, Jung JH (2000) New acetylenic compounds from the stony coral *Montipora* sp. *J Nat Prod* 63:1511–1514
- Baird AH, Pratchett MS, Gibson DJ, Koziumi N, Marquis CP (2001) Variable palatability of coral eggs to a planktivorous fish. *Mar Freshwater Res* 52:865–868
- Bowden BF, Coll J, Tapiolas DM, Willis RH (1985) Some chemical aspects of spawning in alcyonacean corals. In: *Proceedings 5th international coral reef Congress*, vol 4. pp 325–330
- Ciereszko LS (1989) Sterol and diterpenoid production by zooxanthellae in coral reefs: a review. *Biol Ocean* 6:363–374
- De Nys R, Steinberg PD, Willemsen P, Dworjanyn SA, Gabelish CL, King RJ (1995) Broad spectrum effects of secondary metabolites from the red alga *Delisea pulchra* in antifouling assays. *Biofouling* 8:259–271
- Fearon RJ, Cameron AM (1996) Larvotoxic extracts of the hard coral *Goniopora tenuidens*: allelochemicals that limit settlement of potential competitors? *Toxicon* 34:361–367
- Fusetani N, Toyoda T, Asai N, Matsunaga S, Maruyama T (1996) Montiporic acids A and B, cytotoxic and antimicrobial polyacetylene carboxylic acids from eggs of the scleractinian coral *Montipora digitata*. *J Nat Prod* 59:796–797
- Gil-Turnes MS, Fenical W (1992) Embryos of *Homarus americanus* are protected by epibiotic bacteria. *Biol Bull* 182:105–108
- Givskov M, De Nys R, Manefield M, Gram L, Maximilien R, Eberl L, Molin S, Steinberg PD, Kjelleberg S (1996) Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. *J Bacteriol* 178(22):6618–6622
- Gregersen T (1978) Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* 5:123–127
- Gunthorpe L, Cameron AM (1990a) Intracolony variation in toxicity in scleractinian corals. *Toxicon* 28:1221–1227
- Gunthorpe L, Cameron AM (1990b) Toxic exudate from the hard coral *Goniopora tenuidens*. *Toxicon* 28:1347–1350
- Gunthorpe L, Cameron AM (1990c) Widespread but variable toxicity in scleractinian corals. *Toxicon* 28:1199–1219
- Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) *Coral reefs*. Elsevier, Amsterdam, pp 133–207
- Harvell CD, West JM, Griggs C (1996) Chemical defense of embryos and larvae of a West Indian Gorgonian coral, *Briareum Asbestinum*. *Invert Reprod Dev* 30(1–3):239–247
- Hodgson G (1990) Tetracycline reduces sedimentation damage to corals. *Mar Biol* 104(3):493–496
- Holmström C, Kjelleberg S (1999) Marine *Pseudoalteromonas* species are associated with higher organisms and produce biologically active extracellular agents. *FEMS Microbiol Ecol* 4:285–293
- Holmström C, James S, Neilan BA, White DC, Kjelleberg S (1998) *Pseudoalteromonas tunicata* sp nov a bacterium that produces antifouling agents. *Int J Syst Bacteriol* 48:1205–1212
- Holmström C, Egan S, Franks A, McCloy S, Kjelleberg S (2002) Antifouling activities expressed by marine surface associated *Pseudoalteromonas* species. *FEMS Microbiol Ecol* 41:47–58
- Jensen P, Harvell C, Wirtz K, Fenical W (1996) Antimicrobial activity of extracts of Caribbean gorgonian corals. *Mar Biol* 125:411–419
- Kelman D, Kushmaro A, Loya Y, Kashman Y, Benayahu Y (1998) Antimicrobial activity of a Red Sea soft coral, *Parerythropodium fulvum*: reproductive and developmental considerations. *Mar Ecol Prog Ser* 169:87–95
- Koh EGL (1997) Do scleractinian corals engage in chemical warfare against microbes? *J Chem Ecol* 23:379–398
- Kushmaro A, Loya Y, Fine M, Rosenberg E (1996) Bacterial infection and coral bleaching. *Nature* 380(6573):396
- Kushmaro A, Banin E, Loya Y, Stackebrandt E, Rosenberg E (2001) *Vibrio shiloi* sp nov., the causative agent of bleaching of the coral *Oculina patagonica*. *Int J Syst Evol Micro* 51:1383–1388
- Lindquist N (2002) Chemical defense of early life stages of benthic marine invertebrates. *J Chem Ecol* 28:1987–2000
- Maximilien R, de Nys R, Holmstrom C, Gram L, Givskov M, Crass K, Kjellebeger S, Steinberg PD (1998) Chemical mediation of bacterial surface colonisation by secondary metabolites from the red alga *Delisea pulchra*. *Aquat Microb Ecol* 15(3):233–246
- Mitchell R, Chet I (1975) Bacterial attack of corals in polluted seawater. *Microb Ecol* 2:227–233
- Pratchett MS, Baird AH, Marquis CP (2002) Comparative palatability of coral eggs. In: *Proceedings of 9th international coral reef symposium*, vol 1. pp 391–394
- Russell AD, Quesnel LB (1983) *Antibiotics: assessment of antimicrobial activity and resistance*. Academic, London
- Segal LA, Ducklow HW (1982) A theoretical investigation into the influence of sublethal stresses on coral-bacterial ecosystem dynamics. *Bull Mar Sci* 32(4):919–935
- Slattery M, McClintock JB, Heine JN (1995) Chemical defenses in Antarctic soft corals: evidence for antifouling compounds. *J Exp Mar Biol Ecol* 190(1):61–77
- Steinberg PD, de Nys R, Kjelleberg S (2001) Chemical mediation of surface colonization in *Marine Chemical Ecology*. In: McClintock JB, Baker BJ (eds) *CRC Press*, Boca Raton, pp 355–387
- Steinberg PD, de Nys R, Kjelleberg S (2002) Chemical cues for surface colonization. *J Chem Ecol* 28(10):1935–1951
- Willis BL, Babcock RC, Harrison PL, Oliver JK (1985) Patterns in the mass spawning of corals on the Great Barrier Reef from 1981 to 1984. In: *Proceedings of 5th international coral reef congress*, vol 4. pp 343–348
- Zar JH (1999) *Biostatistical analysis*, 4th edn. Prentice Hall, Upper Saddle River