

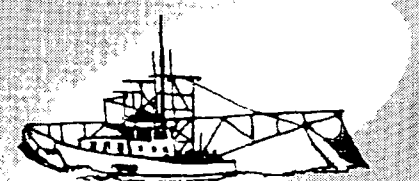
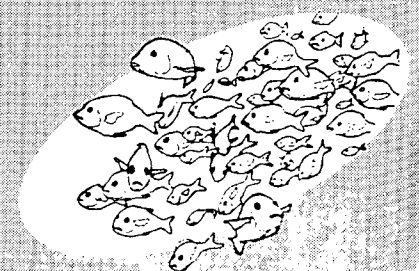
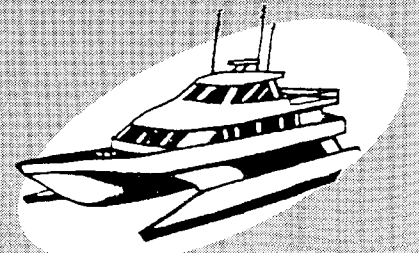
CRC REEF RESEARCH

TECHNICAL REPORT

Dissolved free amino acid concentration: implications for COTS larval nutrition

Tenshi Ayukai, Diane Miller and Lynn Swann

Australian Institute of Marine Science (A.I.M.S.)



Project Funded by the CRC Reef Research Centre

This project is part sponsored by the Environment Management Charge (EMC)
paid by operators in the Great Barrier Reef Marine Park

CRC REEF RESEARCH TECHNICAL REPORT

DISSOLVED FREE AMINO ACID CONCENTRATION: IMPLICATIONS FOR COTS LARVAL NUTRITION

Tenshi Ayukai, Diane Miller, and Lynn Swann

Australian Institute of Marine Science
PMB No.3, Townsville M.C.
Queensland 4810

A report funded by the CRC Reef Research Centre.

The CRC Reef Research Centre was established under the Australian Government's Cooperative Research Centres Program.

The Centre, established in 1993, undertakes an integrated program of applied research and development, training and education, aimed at increasing opportunities for ecologically sustainable development of the Great Barrier Reef and providing an improved scientific basis for Reef management and regulatory decision making.

CRC Reef Research Centre
c/- James Cook University
TOWNSVILLE QLD 4811
Phone: (077) 81 4976
Fax: (077) 81 4099
Email: crc.reef@jcu.edu.au

©Cooperative Research Centre for Ecologically Sustainable Development of the Great Barrier Reef

National Library of Australia Cataloguing-in-Publication entry

Ayukai, T. (Tenshi), 1957 - .

Dissolved free amino acid concentration: implications for COTS larval nutrition.

Bibliography.

Includes index.

ISBN 1 876054 08 5.

1. Nutrient pollution of water - Queensland - Great Barrier Reef. 2. Crown-of-thorns starfish - Larvae - Queensland - Great Barrier Reef. 3. Coral reef ecology - Queensland - Great Barrier Reef. I. Miller, Diane (Diane Louise). II. Swann, Lynn (Lynelle). III. Cooperative Research Centre for Ecologically Sustainable Development of the Great Barrier Reef (Australia). IV. Title. (Series: CRC Reef Research technical report; 9).

593.93

This publication should be cited as:

Ayukai, T., Miller, D., & Swann, L. (1996)

Dissolved free amino acid concentration: implications for COTS larval nutrition.

CRC Reef Research Centre

Technical Report No. 9

Townsville; CRC Reef Research Centre, 30 pp.

This work is copyright. The Copyright Act 1968 permits fair dealing for study, research, news reporting, criticism or review. Selected passages, tables or diagrams may be reproduced for such purposes provided acknowledgement of the source is included. Major extracts of the entire document may not be reproduced by any process without written permission of the Director, CRC Reef Research Centre.

Published by the Cooperative Research Centre for Ecologically Sustainable Development of the Great Barrier Reef.

Further copies may be obtained from CRC Reef Research Centre, c/- James Cook University Post Office, Townsville, QLD 4811.

Printed by James Cook University of North Queensland.

TABLE OF CONTENTS

1. Objectives and Context	1
2. Introduction	2
3. Materials and methods.....	3
4. Results and discussion.....	7
5. Acknowledgements	13
6. References.....	14
7. Appendix	18

LIST OF TABLES

Table 1: Ambient concentrations of dissolved free amino acids (μM) in tropical and subtropical waters	3
Table 2: Correspondence between sampling dates and stations, the number of samples collected and the level of terrestrial runoff of freshwater.....	6
Table 3: Range of ambient concentrations of dissolved free amino acids (μM) in each of 17 field trips to the Cairns Section	8

LIST OF FIGURES

Figure 1: Location of sampling sites in the Cairns Section of the GBR.....	5
Figure 2: Frequency distribution of concentrations of dissolved free amino acids (DFAA) in the Cairns Section during flood and non-flood periods	9
Figure 3: Relationship between concentrations of dissolved free amino acids (DFAA) and distance from the shore in the Cairns Section during flood and non-flood periods	10

EXECUTIVE SUMMARY

Understanding the causes of crown-of-thorns starfish outbreaks is critical to the effective management of the Great Barrier Reef Marine Park. If managers are to control outbreaks on a large scale it can be only through tackling the causes - not the symptoms. Indeed, knowing the causes (in particular the role of human activities) will determine whether or not management intervention is philosophically and ecologically appropriate.

Two main theories to explain crown-of-thorns starfish outbreaks remain popular and, as yet, unresolved. One of these relates to the possible increase in food for larval starfish resulting from increases in nutrient levels due to freshwater run-off from adjacent land masses. Logically, the additional supply of food to starfish larvae would result in greater survival either directly (if larvae are normally food-limited) or indirectly by accelerating development rates. Research at the Australian Institute of Marine Science is investigating these possibilities.

The substantial quantities of nutrients contained in terrestrial run-off often lead to increases in phytoplankton - the primary food source of larval crown-of-thorns starfish. While this occurs naturally as a result of cyclones and torrential rain, modification of catchments and coastal plains for agriculture and development could enhance the effects.

Crown-of-thorns starfish larvae appear to have alternative sources of nutrition, in particular dissolved free amino acids (DFAA). Previous research has shown that crown-of-thorns starfish larvae are rather exceptional in their ability to take up DFAA. However, concentrations of amino acids in coral reef waters under normal conditions are generally too low to make significant contributions to the larval diet (if indeed DFAA is taken up for nutritional purposes). Whether or not these background concentrations changed seasonally, particularly in response to terrestrial run-off, was unknown.

The project by Dr Tenshi Ayukai, Diane Miller and Lynn Swan monitored the levels of DFAA over the crown-of-thorns starfish spawning period (when larvae were most likely to be present) in an area of known increasing populations of the starfish. A cyclone with localised torrential rainfalls occurred in the area during the study.

The results of the study showed that DFAA concentrations were not affected by terrestrial run-off. Throughout the starfish's spawning season amino acid concentrations remained at levels where this potential food source would not play a significant role in larval nutrition.

Further research into this theory of links between larval nutrition and starfish outbreaks should concentrate on the dynamics of the primary food source - phytoplankton.

Dr Brian Lassig

Coordinator

Great Barrier Reef Marine Park Authority COTS Program

1. OBJECTIVES AND CONTEXT

Task objectives:

- (1) To measure the variation in background concentration of dissolved free amino acids during the spawning season of crown-of-thorns starfish.
- (2) To determine the magnitude to which the concentration of dissolved free amino acids is affected by nutrient inputs through episodic events, including heavy terrestrial runoff.

Summary:

The analysis of dissolved free amino acids (DFAA) was conducted for 197 samples, which were collected in the Cairns Section of the Great Barrier Reef Marine Park on 17 separate occasions during the summers of 1992/93, 1993/94 and 1994/95. Of these samples, 86 were collected following the passage of a tropical cyclone (Sadie in January 1994) and localized torrential rainfalls. There was no significant difference in ambient DFAA concentration between flood and non-flood periods, with average values of $0.111 \pm 0.047 \mu\text{M}$ and $0.109 \pm 0.041 \mu\text{M}$ ($x \pm 1sd$), respectively.

The larvae of crown-of-thorns starfish (COTS) have an ability to take up DFAA (Hoegh-Guldberg 1994). Ambient DFAA concentrations are, however, at least an order of magnitude lower than the concentration at which COTS larvae are able to gain sufficient DFAA to meet their metabolic expenditure ($1.8 \mu\text{M}$ at the bipinnaria stage and $4.7 \mu\text{M}$ at the brachiolaria stage, Hoegh-Guldberg 1994). In other words, the contribution of DFAA to the nutrition of COTS larvae in the field is no more than 10 % of their metabolic expenditure.

Management implications:

The terrestrial runoff of freshwater brings a sizeable amount of nutrients into the sea and often triggers phytoplankton blooms. Such an event has been well-documented for GBR waters (e.g. Revelante and Gilmartin 1982, Furnas 1989) and its impact on the nutrition of COTS larvae has been discussed (e.g. Brodie 1992, Ayukai 1993). Little attention was, however, paid towards a possible increase in non-phytoplankton food for COTS larvae. This study suggests no apparent link between terrestrial runoff of freshwater and dynamics of DFAA, of which the potential importance in the nutrition of COTS larvae has been suggested (Hoegh-Guldberg 1994).

2. INTRODUCTION

Transepidermic uptake of dissolved free amino acids (DFAA) has been reported for a wide range of soft-bodied marine invertebrates (Stephens 1988, Manahan 1990). Intracellular DFAA concentrations in marine invertebrates can be as high as 100 mM (e.g. Davies and Stephens 1984a, b), whereas ambient DFAA concentrations are usually below several hundred nM (e.g. Lee and Bada 1975, 1977, Mopper and Lindroth 1982). Hence, the DFAA gradient across the epidermis is in the order of 10^6 and DFAA must be taken up *via* active transport processes (Stephens 1988). According to Stephens (1988), DFAA are electrogenically co-transported across the epidermis with sodium (as cation complexes with sodium). This process is often accompanied by the release of potassium and/or other molecules into the water. DFAA uptake by echinoderm larvae, for example, results in the loss of glycine, which accounts for more than 90 % of intracellular DFAA and is known to function as an osmolyte (Davies and Stephens 1984a, b). In the case of marine algae, uptakes of three types of DFAA, neutral, acidic and basic, appear to involve separate sodium co-transport systems, although the presence of a general DFAA transport system has not totally been denied (Antia et al. 1991). The energetic cost for DFAA uptake is still to be investigated, but may be substantial (Antia et al. 1991).

The availability of DFAA to marine invertebrates in the field has been in question for some time (Stephens 1988). The half saturation constant of DFAA uptake in bacteria is as low as several nM (e.g. Fuhrman and Ferguson 1986, Fuhrman 1990, Suttle et al. 1991), at least two orders of magnitude lower than those in marine invertebrates (Stephens 1988). In addition, bacteria can quickly respond to the increase in ambient DFAA concentration by multiplying their populations. Hence, bacteria usually outcompete marine invertebrates.

The possible importance of DFAA in the nutrition of the larvae of crown-of-thorns starfish (COTS) has been suggested by some workers (Lucas 1982, Olson and Olson 1989) and laboratory studies have shown that COTS larvae are indeed able to take up DFAA (Hoegh-Guldberg 1994). In common with other tropical waters, however, ambient DFAA concentrations in Great Barrier Reef (GBR) waters remain below a few hundred nM (Table 1) and seem too low to make any significant contribution to the nutrition of COTS larvae (Ayukai 1993). The existing data on ambient DFAA concentrations in GBR waters, however, originate from snap-shot sampling designs and do not resolve the variability of ambient DFAA concentrations during the spawning season of COTS (November - January, Babcock and Mundy 1992). In particular, little is known about the magnitude to which ambient DFAA

concentrations are affected by nutrient inputs through episodic events, such as the heavy terrestrial runoff of freshwater.

The answer to the above-mentioned question is pertinent to Birkeland's "terrestrial runoff hypothesis", which suggests that the terrestrial runoff of freshwater brings a sizeable amount of nutrients into the sea and that this relaxes the food limitation in COTS larvae, leading to higher recruitment success and to the establishment of an outbreak population. Although the terrestrial runoff hypothesis is one of the natural causes hypotheses, its evaluation bears importance to the concern over the effect of increasing nutrient inputs from anthropogenic sources on the COTS population dynamics (Brodie 1992).

Table 1. Ambient concentrations of dissolved free amino acids (μM) in tropical and subtropical waters.

<i>Oceanic waters</i>		
Equatorial Pacific	0.02 - 0.06	Lee and Bada (1975)
Sargasso Sea	0.01 - 0.04	Lee and Bada (1977)
	0.02 - 0.15	Liebezeit et al. (1980)
	trace - 0.06	Suttle et al. (1991)
GBR	trace - 0.07	Ayukai and Hoegh-Guldberg (1992)
	trace - 0.16	Ayukai et al. (1993)
<i>Coastal and reefal waters</i>		
Bermuda	trace - 0.22	Ferrier (1991)
Biscayne Bay, Florida	0.02 - 0.05	Lee and Bada (1977)
Mombasa, Kenya*	0.86 - 1.07	Schlickter and Liebezeit (1991)
Myrmidon Reef, GBR	trace - 0.17	Ayukai and Hoegh-Guldberg (1992)

* Under the influence of major sewage discharges from the urban area.

3. MATERIALS AND METHODS

Sampling sites and procedures

Forty three stations were sampled during 17 field trips to the Cairns Section of the GBR Marine Park during the summers of 1992/39, 1993/94 and 1994/95 (Fig. 1). Correspondence between sampling dates and sites are shown in Table 2. Eighty six of the total 197 DFAA samples were collected 1 - 7 days after heavy rainfalls caused by the passage of a tropical cyclone (Sadie in January 1994) or a monsoonal activity. Unfortunately, no reliable salinity data were obtained in most of the samplings along Cairns (Stns. A - E) and Port Douglas (Stns. F - I) transects, because of the malfunction of the YSI conductivity meter used.

At Stns. A - I, water samples were usually collected from just below the sea surface (0.5 m) as well as a few meters above the bottom. For the remaining stations (Stns. 1 - 18 m), water samples were collected from 2 - 4 depths, usually at 10 m intervals from the sea surface (see the Appendix for the detail). A 5-l water Niskin bottle or a locally manufactured 3-l bottle used for sampling was acid-washed and rinsed thoroughly with Super-Q water (Millipore) prior to each field trip.

Except for the field trip on 28 February 1995, triplicate 1.5 ml subsamples were withdrawn from each bottle, filtered through a 0.2 μ m or 0.45 μ m disposable syringe filter and stored at -20°C for subsequent DFAA analysis in the laboratory. If not analyzed within several months, DFAA samples were kept in a -80 °C deep freezer. The precautions outlined by Fuhrman and Bell (1985) were followed in sampling and sample storage for minimizing the chance of contamination.

Analytical procedure

DFAA analysis was conducted by high performance liquid chromatography (HPLC), using the pre-column derivatising reagent, *o*-phthaldialdehyde-2-mercaptoethanol (OPA-MCE). The analytical procedure was similar to those reported by Stanley et al. (1987) and Ayukai et al. (1993) (see also Mopper and Lindroth 1982). For the DFAA samples collected after January 1994, the procedure was modified to optimise the resolution of eight DFAA species: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg) and alanine (Ala). These DFAA usually account for more than 90 % of the total (Ayukai et al. 1993). More importantly, the recent study has suggested that COTS larvae are able to take up neutral DFAA, but lack or have a reduced ability to take up acidic and basic DFAA (Okaji, unpublished). The modification of the analytical procedure is unlikely to result in underestimation of DFAA available to COTS larvae in the field.

HPLC-grade tetrahydrofuran (THF) and methanol were obtained from FSE and BDH, respectively. All other analytical-grade chemicals, including OPA, MCE and amino acid standards were obtained from Sigma Aldrich. Super-Q water was used for preparation of all aqueous solutions. The reversed-phase column, Brownlee RP18 (5 μ m particle size, 250 x 4.6 mm) and the guard column (Alltima C18, 5 μ m guard cartridge) were supplied by Alltech.

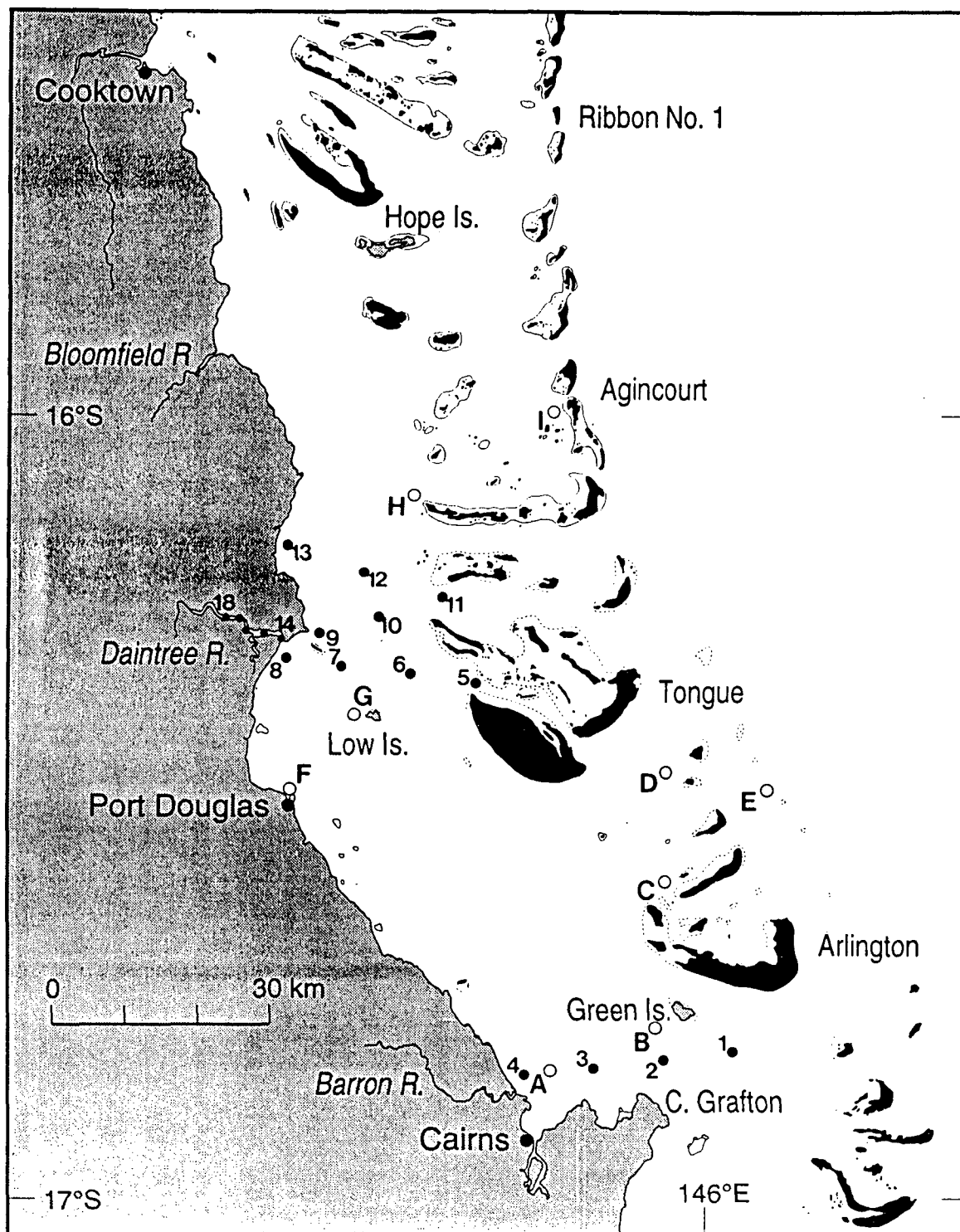


Figure 1: Location of sampling sites in the Cairns Section of the Great Barrier Reef

Table 2. Correspondence between sampling dates and stations, the number of samples collected and the level of terrestrial runoff of freshwater.

Date	Station	No. of samples	Freshwater input	Salinity range (ppt)
<i>Cairns</i>				
17/10/94	A - D	8	low	
08/11	A - E	10	low	
15/12	A - E	10	low	
10/01/95	A - D	10	low	
21/02	A - D	8	high	
<i>Port Douglas</i>				
18/01/94	F - I	8	low	
01/02 (Sadie)	F - I	8	high	22 - 32*
03/02 (Sadie)	F - I	8	high	31 - 33*
12/12	F - I	8	low	
11/01/95	F - I	8	low	
30/01	F - I	8	low	
14/02	F - I	8	high	
09/03	F - I	8	high	
<i>Cairns - Port Douglas</i>				
07-08/12/92	1 - 8	22	low	35 - 36**
07-10/01/93	1 - 8	21	low	35 - 36**
22-23/02/94	1 - 8	21	high	26 - 35**
28/02/95	7 - 18	25 [§]	high	14 - 35**

* Salinity data were provided by the Great Barrier Reef Marine Park Authority. Measured using a YSI conductivity meter.

** Measured using a Seabird CTD profiler.

[§] Incl. 5 samples collected from Daintree River.

The HPLC system consisted of a solvent conditioner (LKB 2156), for degassing solvents with helium, a pressure mixing valve (LKB 2040-203), a mixer driver (LKB 11360-2), a system controller (LKB 2152 LC), a dual piston single pump (LKB 2150), a manual injection valve (Rheodyne 7125) with a 50 µl loop, a column oven (Shimadzu CTO-10A), a fluorescence detector (Shimadzu RF-551) and an integrator (LKB 2221).

The Brownlee RP18 column was eluted at a constant flow rate of 1.0 ml min⁻¹ with solvent A (78:22:1, 0.1M sodium acetate: methanol:THF) and solvent B (methanol). In the original procedure, the column oven was set at 45 °C and the proportion of solvent B was increased from 15 to 50 % in 25 min, 75 % in 40 min and 100 % in 50 min and returned to 0 % at the end of the 60 min run. In the modified procedure, the temperature of the column oven was reduced to 30 °C and the proportion of solvent B was changed as follows: 0 % from 0 - 8 min, 0 - 10 % from 8 - 9 min, 10 % from 9 - 28 min and 10 - 0 % by 29 min. The excitation and emission wavelengths of the fluorescence detector were set at 330 nM and 440 nM, respectively.

The OPA-MCE reagent was prepared by dissolving 25 mg OPA in 0.5 ml methanol, to which 25 µl MCE and 12 ml 0.4 M sodium borate was then added. A hundred µl of the OPA-MCE reagent was mixed with 500 µl of a sample in a 1.5 ml test tubes with cap (Sarstedt, Disposable Products) and allowed to stand for 2 min. Fifty µl of this reagent-sample mixture was then injected to the HPLC system. The same derivatisation procedure was used for standards, standard blanks (0.1 M sodium acetate) and aged seawater blanks.

Treatment of data

Except for one occasion (28 February 1995), triplicate 1.5 ml subsamples were collected for DFAA analysis. The third subsample, however, was not analysed if the results of the first and second subsamples were in agreement (the difference ² 30 % of the mean). If the third subsample was analysed, the average of two nearest values was then calculated. In the case that only duplicate subsamples were available for DFAA analysis and an extremely high value occurred in one subsample, it was assumed to be due to contamination and ignored.

4. RESULTS AND DISCUSSION

The lowest and highest ambient DFAA concentrations observed in this study were 0.04 µM and 0.27 µM, respectively (Table 3). The detection limit (3 s.d.) of the method was

calculated as 0.07 μM , with all values below this level being considered "trace" concentrations. This relatively high detection limit is primarily because of the unavailability of aged seawater blanks with very low levels of DFAA in this study.

Table 3 does not suggest any link between freshwater input and level of ambient DFAA concentrations. This is more obvious in Fig. 2, which compares the frequency distribution of ambient DFAA concentrations during flood periods with that during non-flood periods. For pooled data, the average DFAA concentration during flood periods (0.111 μM) is marginally higher than that during non-flood periods (0.109 μM), but the difference is by no means statistically significant.

Table 3. Range of ambient concentrations of dissolved free amino acids (μM) in each of 17 field trips to the Cairns Section.

Date	Freshwater input	Lowest concentration	Highest concentration
<i>Cairns</i>			
17/10/94	low	0.07	0.23
08/11	low	0.06	0.13
15/12	low	0.07	0.21
10/01/95	low	0.10	0.20
21/02	high	0.07	0.22
<i>Port Douglas</i>			
18/01/94	low	0.10	0.23
01/02	high	0.09	0.17
03/02	high	0.06	0.25
12/12	low	0.05	0.20
11/01/95	low	0.07	0.20
30/01	low	0.10	0.20
14/02	high	0.10	0.20
09/03	high	0.09	0.17
<i>Cairns - Port Douglas</i>			
07-08/12/92	low	0.05	0.14
07-10/01/93	low	0.08	0.19
22-23/02/94	high	0.04	0.10
28/02/95	high	0.05*	0.27

* Incl. 5 samples collected in the Daintree River.

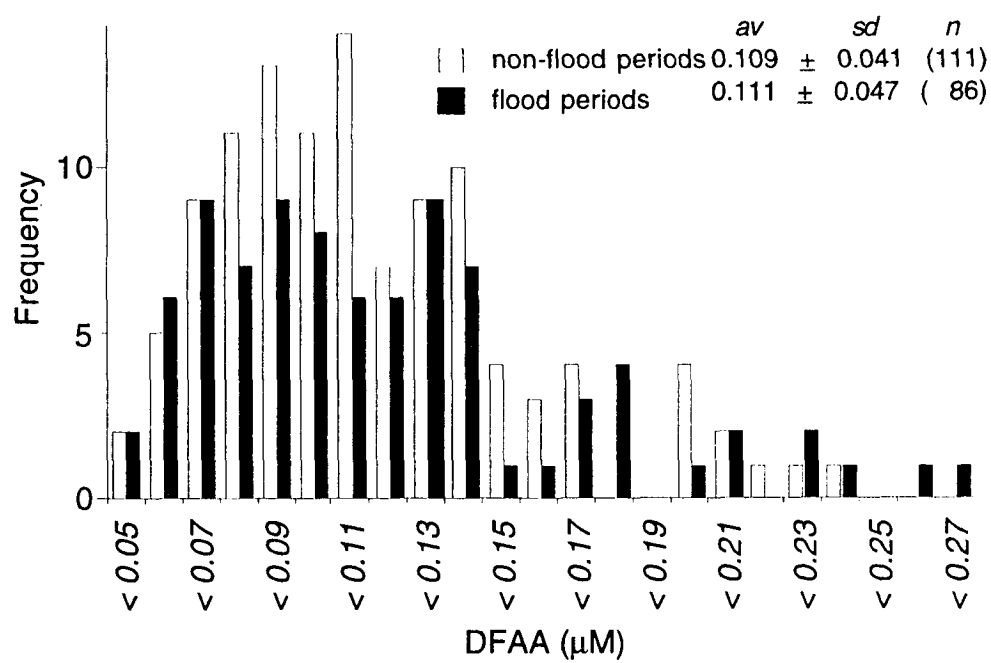


Figure 2: Frequency distribution of concentrations of dissolved free amino acids (DFAA) in the Cairns Section during flood and non-flood periods.

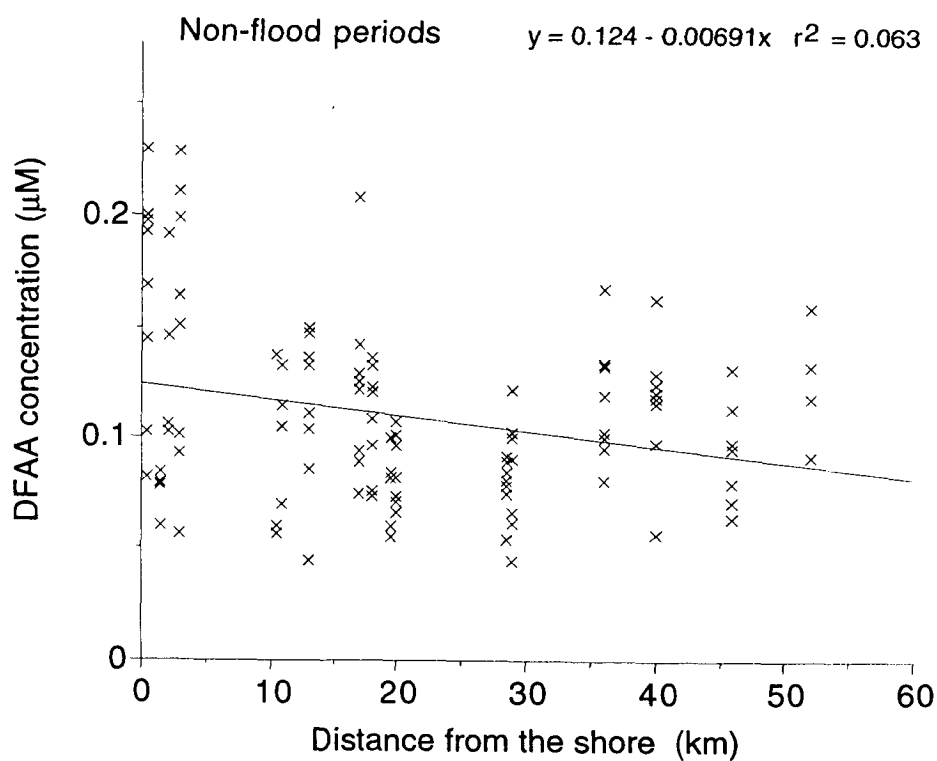
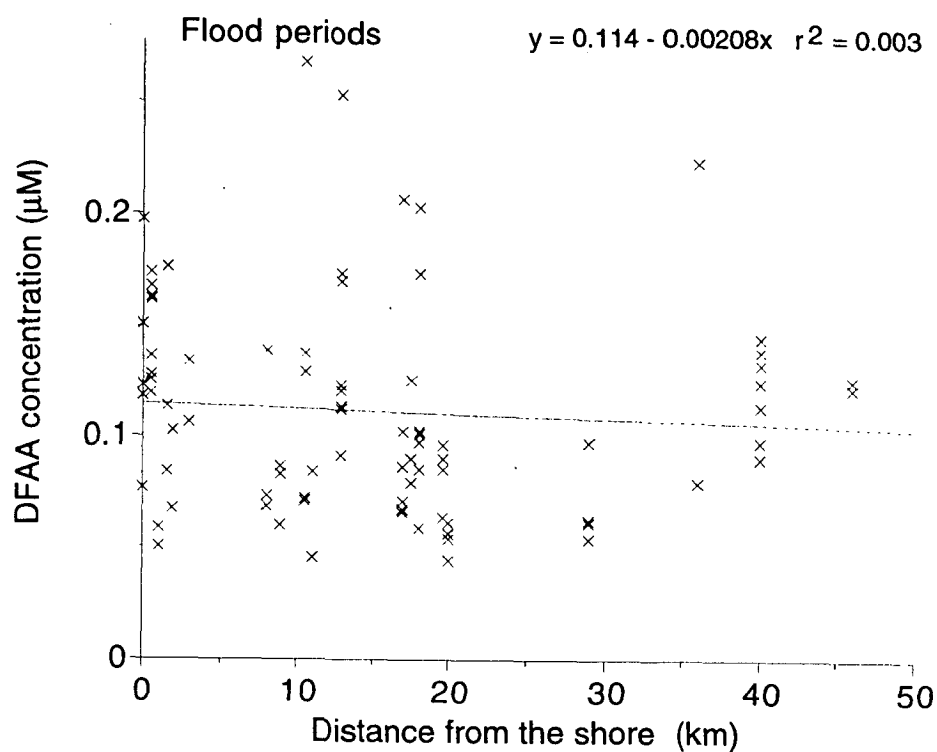


Figure 3: Relationship between concentrations of dissolved free amino acids (DFAA) and distance from the shore in the Cairns Section during flood and non-flood periods.

During non-flood periods, relatively high ambient DFAA concentrations tend to occur at stations closer to the coast (Fig. 3 bottom). The slope of the regression line, however, does not significantly differ from zero (ANOVA, $p > 0.05$). During flood periods, there appears to be a small increase in ambient DFAA concentration in mid-shelf areas (Fig. 3_{top}, 10 - 20 km from the coast). This does not, however, alter the view that freshwater inputs do not have a significant impact on the distribution of DFAA concentrations.

Ambient DFAA concentrations in river waters, although the data are very limited ($n = 5$), are relatively low, ranging between 0.08 and 0.20 μM (see Appendix). A sizeable amount of DFAA may be generated during the degradation of riverine organic matter. As mentioned earlier, however, bacteria have an extremely high affinity with DFAA and are able to keep ambient DFAA concentrations in check (e.g. Fuhrman and Ferguson 1986, Fuhrman 1990, Suttle et al. 1991). Seeing no influence of freshwater inputs on ambient DFAA concentrations and their spatial distribution is not surprising.

Ambient DFAA concentrations measured in this study (0.04 - 0.27 μM) are slightly higher than those reported for GBR waters (trace - 0.17 μM) and other tropical waters (trace - 0.22 μM , Table 1). As far as COTS larvae's nutrition is concerned, however, the difference of this magnitude bears little importance. Specifically, ambient DFAA concentrations are one order of magnitude lower than the compensating concentration, at which the energy intake of COTS larvae from DFAA balances with their metabolic expenditure (1.8 μM at the bipinnaria stage and 4.7 μM at the brachiolaria stage, Hoegh-Guldberg 1994). In other words, the contribution of DFAA to the nutrition of COTS larvae in the field is no more than 10 % of their metabolic expenditure under normal circumstances.

Dissolved organic matter (DOM) is the biggest reservoir of organic carbon in the ocean and the third biggest reservoir on earth (Hedges 1992). These notions have often created an impression that the contribution of DOM to the nutrition of marine invertebrates may be potentially enormous (Jorgensen 1976). The true nature of DOM, however, has been considered as one of the largest unknowns in marine chemistry (Williams 1975, Suzuki et al. 1985). About half of DOM is believed to be in a colloidal phase ($> 1,000$ Dalton, Cauwet 1978). Such high molecular weight DOM needs to be fragmented before uptake and even bacteria with extracellular hydrolytic enzymes are not fully successful in doing so (e.g. Taylor et al. 1985, Brophy and Carlson 1989). A significant fraction of low molecular weight DOM may also be biologically inert (Amon and Benner 1994). Several classes of low molecular

weight DOM, of which the transepidermic uptake has been reported for marine invertebrates, usually represent less than 5 % of the total (William 1975). Marine invertebrates have a relatively low affinity with DOM other than DFAA (Stephens 1988, Manahan 1990). It is, therefore, important to recognize that DOM is not an unlimited energy source for marine invertebrates.

The terrestrial runoff of freshwater brings a sizeable amount of nutrients into the sea and often triggers phytoplankton blooms. Such an event has been well-documented for GBR waters (e.g. Revelante and Gilmartin 1982, Furnas 1989) and its impact on the nutrition of COTS larvae has been discussed (e.g. Brodie 1992, Ayukai 1993). Little attention was, however, paid towards a possible increase in non-phytoplankton food for COTS larvae. This study suggests no apparent link between terrestrial runoff of freshwater and dynamics of DFAA, of which the potential importance in the nutrition of COTS larvae has been suggested (Hoegh-Guldberg 1994).

5. ACKNOWLEDGEMENTS

We would like to express our special thanks to Mr. D. Baird, Reef Biosearch in Port Douglas, and Mr. M. Short, Queensland Department of Environment in Cairns, for their assistance in collecting DFAA samples. DFAA samples can be very easily contaminated during every handling process. Yet, we have never seen any sign of serious contamination in DFAA samples collected by them.

We would like to thank the captain and crew of the R/Vs Lady Basten, Harry Messel and Sirius for their able assistance at sea. Prof. C. Crossland and Dr. B. Lassig provided us with valuable comments on the sampling design.

This study was supported by the CRC Reef Research Centre and the Great Barrier Reef Marine Park Authority.

6. REFERENCES

- Amon, R.M.W. & R. Benner (1994) 'Rapid cycling of high-molecular-weight dissolved organic matter in the ocean', *Nature*, 369: 549-552.
- Antia, N.J., P.J. Harrison, & L. Oliveira (1991) 'The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology', *Phycologia*, 30: 1-89.
- Ayukai, T. (1993) 'Resource availability to the larvae of the crown-of-thorns starfish in Great Barrier Reef waters: an overview', In U. Engelhardt and B. Lassig (eds.), *The Possible Causes and Consequences of Outbreaks of the Crown-of-thorns Starfish*, Great Barrier Reef Marine Park Authority Workshop Ser. 18., p. 21-35.
- Ayukai, T., H. Sturmey, & L. Swann (1993) 'A preliminary study on the availability of dissolved organic matter to the nutrition of crown-of-thorns starfish larvae', *Final report to the Great Barrier Reef Marine Park Authority*, p. 19.
- Babcock, R.C. & C.N. Mundy (1992) 'Reproductive biology, spawning and field fertilization rates of *Acanthaster planci*', *Aust. J. Mar. Freshwater Res.*, 43: 525-534.
- Birkeland, C. (1982) 'Terrestrial runoff as a cause of outbreaks of *Acanthaster planci* (Echinodermata: Asteroidea)', *Mar. Biol.*, 69: 175-185.
- Brodie, J.E. (1992) 'Enhancement of larval and juvenile survival and recruitment in *Acanthaster planci* from the effects of terrestrial runoff: a review', *Aust. J. Mar. Freshwater Res.*, 43: 539-554.
- Brophy, J.E. & D.J. Carlson (1989) 'Production of biologically refractory dissolved organic carbon by natural seawater microbial populations', *Deep-Sea Res.*, 36: 497-507.
- Cauwet, G. (1978) 'Organic chemistry of seawater particulates: concepts and developments', *Oceanol. Acta*, 1: 99-105.
- Davies, J.P. & G.C. Stephens (1984a) 'Uptake of free amino acids by bacteria-free larvae of the sand dollar *Dendraster excentricus*', *J. Am. Physiol.*, 247: R733-739.

- Davies, J.P. & G.C. Stephens (1984b) 'Regulation of net amino acid exchange in sea urchin larvae (*Strongylocentrotus purpuratus*)', *J. Am. Physiol.*, 247: R1029-1037.
- Ferrier, M.D. (1991) 'Net uptake of dissolved free amino acids by four scleractinian corals', *Coral Reefs*, 10: 183-187.
- Fuhrman, J.A. (1990) 'Dissolved free amino acid cycling in an estuarine outflow plume', *Mar. Ecol. Prog. Ser.*, 66: 197-203.
- Fuhrman, J.A. & T.M. Bell (1985) 'Biological considerations in the measurement of dissolved free amino acids in seawater and implications for chemical and microbiological studies', *Mar. Ecol. Prog. Ser.*, 25: 13-25.
- Fuhrman, J.A. & R.L. Ferguson (1986) 'Nanomolar concentrations and rapid turnover of dissolved free amino acids in seawater: agreement between chemical and microbiological measurements', *Mar. Ecol. Prog. Ser.*, 33: 237-242.
- Furnas, M.J. (1989) 'Cyclonic disturbance and a phytoplankton bloom in a tropical shelf ecosystem', In Okaichi et al. (eds.), *Red Tides: Biology, Environmental Science, and Toxicology*, Elsevier, Amsterdam, p. 273-276.
- Hedges, J.I. (1992) 'Global biogeochemical cycles: progress and problems', *Mar. Chem.*, 39: 67-93.
- Hoegh-Guldberg, O. (1994) 'Uptake of dissolved organic matter by larval stage of the crown-of-thorns starfish *Acanthaster planci*', *Mar. Biol.*, 120: 55-63.
- Jorgensen, C.B. (1976) 'August Putter, August Krogh, and modern ideas on the use of dissolved organic matter in aquatic environments', *Biol. Rev.*, 51: 291-328.
- Lee, C. & J.L. Bada (1975) 'Amino acids in equatorial Pacific Ocean water', *Earth Planet Sci. Lett.*, 26: 61-68.
- Lee, C. & J.L. Bada (1977) 'Dissolved amino acids in the equatorial Pacific, the Sargasso Sea, and Biscayne Bay', *Limnol. Oceanogr.*, 22: 502-510.

- Liebezeit, G., M. Bolter, I.F. Brown, & R. Dawson (1980) 'Dissolved free amino acids and carbohydrates at pycnocline boundaries in the Sargasso Sea and related microbial activity', *Oceanogr. Acta*, 3: 357-362.
- Lucas, J.S. (1982) 'Quantitative studies of feeding and nutrition during larval development of the coral reef asteroid *Acanthaster planci* (L)', *J. Exp. Mar. Biol. Ecol.*, 65: 173-193.
- Manahan, D.T. (1990) 'Adaptations by invertebrate larvae for nutrient acquisition from seawater', *Am. Zool.*, 30: 147-160.
- Mopper, K. & P. Lindroth (1982) 'Diel and depth variations in dissolved free amino acids and ammonium in the Baltic Sea determined by shipboard HPLC analysis', *Limnol. Oceanogr.*, 27: 336-347.
- Olson, R.R. & M.H. Olson (1989) 'Food limitation of planktotrophic marine invertebrate larvae: Does it control recruitment success?', *Annu. Rev. Ecol. Syst.*, 20: 225-247.
- Revelante, N. & M. Gilmartin (1982) 'Dynamics of phytoplankton in the Great Barrier Reef lagoon', *J. Plankton Res.*, 4: 47-76.
- Schlickter, D. & G. Liebezeit (1990) 'The natural release of amino acids from the symbiotic coral *Heteroxenia fuscescens* (Ehrb) as a function of photosynthesis', *J. Exp. Mar. Biol. Ecol.*, 150: 83-90.
- Stanley, S.O., K.G. Boto, D.M. Alongi, & F.T. Gillan (1987) 'Composition and bacterial utilisation of free amino acids in tropical mangrove sediments', *Mar. Chem.*, 22: 13-30.
- Stephens, G.C. (1988) 'Epidermal amino acid transport in marine invertebrates', *Biochim. Biophys. Acta*, 947: 113-138.
- Suttle, C.A., A.M. Chan & J.A. Fuhrman (1991) 'Dissolved free amino acids in the Sargasso Sea: uptake and respiration rates, turnover times, and concentrations', *Mar. Ecol. Prog. Ser.*, 70: 189-199.
- Suzuki, Y., Y. Sugimura & T. Itoh (1985) 'A catalytic oxidation method for the determination of total nitrogen dissolved in seawater', *Mar. Chem.*, 16: 83-97.

Taylor, G.T., R. Iturriaga & C.W. Sullivan (1985) 'Interactions of bacterivorous grazers and heterotrophic bacteria with dissolved organic matter', *Mar. Ecol. Prog. Ser.*, 23: 129-141.

Williams, P.J. leB. (1975) 'Biological and chemical aspects of dissolved organic material in seawater', In: J. P. Riley, G. Skirrow (eds), *Chemical Oceanography*, Vol. 2, Academic Press, New York, p.301-363.

7. APPENDIX

Data sheets:

Measurement of dissolved free amino acid concentration in the Cairns - Port Douglas area between 1992 and 1995.

Appendix p.1

Depth											Average total
Stn.	(m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	concn. (nM)
Cairns											
Date: 17 October, 1994											
A	0	11	19	44	23	62	17	9	21	206	
		15	30	59	22	69	20	6	30	251	<u>229</u>
	bottom	10	17	25	12	30	12	0	12	118	
		6	17	19	7	25	5	0	4	83	101
B	0	11	11	34	9	41	12	0	14	132	
		10	23	32	13	46	10	0	17	151	142
	bottom	7	17	15	10	30	5	0	6	90	
		5	13	13	6	39	6	0	6	88	89
C	0	5	9	21	6	36	10	0	8	95	
		6	21	23	6	37	5	0	6	104	100
	bottom	7	14	22	9	23	7	0	8	90	
		6	16	16	5	18	6	0	5	72	81
D	0	7	10	14	11	36	12	0	13	103	
		8	17	16	9	37	14	5	16	122	113
	bottom	5	8	14	7	21	6	0	1	62	
		5	12	14	14	23	7	0	5	80	<u>71</u>

Date: 8 November, 1994

A 0	7	29	13	12	17	7	12	12	109	
	5	19	15	5	19	7	0	6	76	93
bottom	5	8	15	3	13	2	0	6	52	
	4	14	12	6	14	3	0	8	61	57
B 0	6	13	16	2	44	6	0	7	94	
	6	10	15	4	44	7	0	8	94	94
bottom	9	14	16	3	26	7	0	8	83	
	7	12	10	0	26	4	0	8	67	75
C 0	10	21	22	9	39	12	0	11	124	
	12	16	25	9	55	11	0	11	139	<u>132</u>
bottom	6	16	18	5	45	9	0	11	110	
	7	10	11	4	50	3	0	9	94	102
D 0	4	16	9	6	26	2	0	4	67	
	4	17	5	9	24	0	0	2	61	<u>64</u>
bottom	8	26	20	7	26	5	0	10	102	
	4	10	12	6	18	3	0	5	58	80
E 0	9	19	24	23	41	8	0	23	147	
	9	12	12	3	60	3	0	20	119	133
bottom	5	15	10	5	22	3	0	11	71	
	7	19	20	14	36	6	0	11	113	92

Appendix p.2

Stn.	Depth (m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	Average total concn. (nM)
Cairns											
<u>Date: 15 December, 1994</u>											
A	0	33	56	36	27	40	15	7	20	234	
		27	17	39	19	50	19	0	16	187	<u>211</u>
	bottom	26	15	29	19	32	15	0	13	149	
		24	16	29	16	34	16	0	18	153	151
B	0	31	15	46	18	52	22	5	28	217	
		28	60	26	16	43	11	0	16	200	209
	bottom	9	14	35	12	43	10	0	18	141	
		6	11	25	6	33	8	0	14	103	122
C	0	8	18	19	12	35	6	0	15	113	
		11	22	28	16	46	8	0	23	154	134
	bottom	8	25	30	16	39	5	0	14	137	
		7	13	21	12	32	6	0	9	100	119
D	0	5	7	13	8	27	3	0	7	70	
		6	6	13	8	27	3	0	8	71	<u>71</u>
	bottom	8	11	18	14	31	6	0	7	95	
		8	10	22	9	32	6	0	7	94	95
E	0	10	17	38	14	52	10	0	16	157	
		13	15	39	10	53	11	0	20	161	159
	bottom	9	20	27	12	46	8	0	12	134	
		6	12	24	5	39	6	0	10	102	118
 <u>Date: 10 January, 1995</u>											
A	0	8	37	25	20	48	7	2	11	158	
		8	28	35	20	56	9	0	13	169	164
	bottom	12	28	49	18	88	13	6	24	238	
		9	18	27	15	67	8	3	13	160	<u>199</u>
B	0	10	8	39	16	51	10	0	14	148	
		6	28	20	11	34	5	0	6	110	129
	bottom	6	11	24	12	38	6	0	7	104	
		8	43	20	11	47	5	0	11	145	125
C	0	5	11	16	11	33	4	0	6	86	
		5	9	22	11	43	5	0	9	104	<u>95</u>
	bottom	10	18	27	13	56	8	0	10	142	
		10	26	36	16	73	10	3	17	191	167
D	0	9	9	29	14	44	7	0	10	122	
		11	14	33	17	45	9	0	10	139	131
	bottom	6	9	18	12	29	6	0	4	84	
		9	32	14	11	32	7	0	7	112	98

Appendix p.3

Stn.	Depth (m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	Average total concn. (nM)
Cairns											
<u>Date: 21 February, 1995</u>											
A	0	6	10	20	11	36	6	0	4	93	
		7	12	29	13	42	7	0	9	119	106
	bottom	10	43	13	12	38	6	0	10	132	
B		12	26	24	9	39	9	3	13	135	134
	0	4	19	14	5	25	3	0	6	76	
		4	16	8	4	21	2	0	3	58	67
C	bottom	4	15	12	5	32	3	0	4	75	
		3	13	9	4	20	2	0	5	56	66
	0	17	33	26	11	47	20	30	26	210	
D		35	14	47	12	67	14	0	49	238	224
	bottom	4	20	8	8	36	3	5	4	88	
		4	7	8	9	32	0	3	6	69	79
D	0	8	21	29	9	34	10	3	15	129	
		8	15	27	9	35	9	0	12	115	122
	bottom	11	27	37	17	35	13	0	21	161	
		6	13	20	7	23	7	2	11	89	125

Appendix p.4

Stn.	Depth (m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	Average total concn. (nM)
Port Douglas											
<u>Date: 18 January, 1994</u>											
F	0	9	18	21	4	78	8	6	24	168	
		12	15	19	2	54	3	4	13	122	145
	bottom	13	27	36	3	107	9	8	18	221	
		16	14	28	8	93	11	2	67	239	230
G	0	11	12	17	5	89	7	7	15	163	
		10	7	18	1	35	8	0	22	101	132
	bottom	4	6	25	3	68	5	3	3	117	
		6	18	14	5	32	6	5	5	91	104
H	0	8	14	18	0	77	12	6	14	149	
		5	13	9	2	58	6	4	26	123	136
	bottom	8	14	16	0	46	3	8	11	106	
		10	8	27	7	72	5	8	20	157	132
I	0	14	12	18	2	54	9	2	9	120	
		6	7	17	1	35	10	4	32	112	116
	bottom	5	5	20	4	67	4	3	14	122	
		8	6	10	2	83	5	2	19	135	129

Date: 1 February, 1994

F	0	7	13	15	2	56	7	3	28	131	
		13	5	18	10	66	7	4	17	140	136
	bottom	9	13	24	5	46	8	11	19	135	
		12	16	10	3	52	5	3	14	115	125
G	0	7	15	12	3	69	7	0	10	123	
		5	7	10	2	27	15	3	34	103	113
	bottom	16	18	24	3	71	11	6	13	162	
		9	12	21	8	92	6	4	31	183	173
H	0	10	5	13	2	39	2	0	8	79	
		6	8	15	2	30	5	4	23	93	86
	bottom	6	7	17	3	55	3	2	8	101	
		11	4	16	5	41	7	0	16	100	101
I	0	17	5	15	4	63	5	3	12	124	
		12	2	9	2	54	8	2	35	124	124
	bottom	10	7	12	5	31	0	4	8	77	
		18	5	10	7	46	7	8	15	116	97

Appendix p.5

Stn.	Depth (m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	Average total concn. (nM)
------	--------------	-----	-----	-----	-----	-----	-----	-----	-----	-------	------------------------------

Port Douglas

Date: 3 February, 1994

F	0	8	27	47	11	68	10	2	3	176	173
		11	11	52	10	71	12	0	2	169	
	bottom	7	14	28	5	33	6	0	8	101	119
10		7	43	25	48	0	4	0	137		
18		25	12	8	94	15	5	46	223		
G	0	27	18	25	14	112	13	7	69	285	<u>254</u>
		12	10	13	5	48	13	2	21	124	
	bottom	8	15	20	6	28	5	0	17	99	112
7		2	8	0	21	3	3	8	52		
5		2	10	0	31	2	5	10	65	<u>59</u>	
bottom	6	7	17	3	55	3	2	8	101		
	11	4	16	5	41	7	0	16	100		101
	I	0	15	10	24	25	40	21	2	32	
4			5	16	8	36	7	5	14	95	
bottom		8	5	23	7	39	2	7	12	103	
	4	6	31	15	44	5	8	9	122	113	

Date: 12 December, 1994

F	0	17	24	35	11	66	11	7	32	203	<u>193</u>
		15	21	28	6	67	12	7	26	182	
	8	6	10	11	0	45	7	6	22	107	103
9		10	19	0	46	7	5	3	99		
8		10	22	5	55	6	4	14	124		
G	0	13	13	40	6	83	12	8 *	175	150	
		15	3	2	5	0	26	1	0		37
	bottom	3	3	12	0	32	3	0	0	53	<u>45</u>
7		12	22	9	52	6	8	13	129		
5		11	15	0	44	5	2	35	117	123	
H	0	3	5	13	3	25	4	1	6		60
		6	5	20	6	31	7	4	13		92
	I	0	7	10	26	8	33	7	8	38	137
5			9	17	2	28	5	1	37	104	121
25		3	4	12	2	28	4	0	13	66	
	3	4	9	2	27	3	0	0	48	57	

Appendix p.6

Stn.	Depth (m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	Average total concn. (nM)
Port Douglas											
<u>Date: 11 January, 1995 (* 0.2μ, ** 0.4μ filter)</u>											
F	0 *	10	66	15	9	40	9	4	23	176	
		13	53	35	7	59	13	7	30	217	<u>197</u>
	0 **	5	8	19	0	40	6	0	11	89	
		4	10	15	0	33	5	0	7	74	82
G	0 *	12	15	32	6	45	10	2	21	143	
		10	14	29	7	39	7	3	19	128	136
	0 **	7	7	21	4	34	5	3	11	92	
		4	5	11	0	30	3	6	19	78	85
H	0 *	7	9	18	4	33	6	1	46	124	
		7	9	28	8	44	6	1	14	117	121
	0 **	5	5	15	0	32	4	0	5	66	
		5	5	17	3	33	4	5	9	81	<u>74</u>
I	0 *	6	12	11	0	24	0	0	72	125	
		9	13	22	3	31	5	8	20	111	118
	0 **	6	6	24	9	53	6	3	14	121	
		7	6	21	10	57	6	6	13	126	124

Date: 30 January, 1995 (* 0.2 μ , ** 0.4 μ filter)

F	0 *	11	27	49	11	70	10	2	3	183	
		8	11	46	10	65	12	0	2	154	169
	0 **	14	11	76	12	76	4	3	13	209	
		14	11	63	11	69	3	3	16	190	<u>200</u>
G	0 *	5	10	27	7	59	6	1	26	141	
		7	11	39	8	61	10	5	12	153	147
	0 **	3	6	23	5	54	5	1	11	108	
		3	11	24	5	53	5	0	13	114	111
H	0 *	5	12	29	7	38	4	3	7	105	
		5	14	33	6	40	6	2	7	113	109
	0 **	3	6	25	4	39	4	3	8	92	
		6	6	28	4	41	6	1	7	99	<u>96</u>
I	0 *	6	24	36	5	49	11	2	12	145	
		8	42	40	8	53	10	3	16	180	163
	0 **	8	6	31	3	41	5	2	8	104	
		4	7	26	3	38	4	2	7	91	98

Appendix p.7

Stn.	Depth (m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	Average total concn. (nM)
------	--------------	-----	-----	-----	-----	-----	-----	-----	-----	-------	------------------------------

Port Douglas

Date: 14 February, 1995 (* 0.2 μ , ** 0.4 μ filter)

F	0 *	10	17	45	9	47	9	7	13	157	
		9	16	53	9	51	10	3	16	167	162
	0 **	9	8	54	8	47	10	0	12	148	
G		12	13	63	10	49	12	3	15	177	163
	0 *	10	13	66	12	52	14	2	15	184	
		8	12	51	9	50	11	1	14	156	170
H	0 **	6	6	47	10	49	10	2	13	143	
		4	5	30	6	43	7	2	5	102	123
	0 *	9	16	59	13	57	13	2	18	187	
I		11	17	70	15	62	14	3	28	220	204
	0 **	7	7	35	4	44	7	0	14	118	
		6	5	28	3	34	5	0	6	87	103
I	0 *	9	12	46	8	46	10	1	14	146	
		7	12	42	9	48	9	2	13	142	144
	0 **	6	5	36	8	48	4	2	23	132	
	7	6	40	6	54	10	0	23	146	139	

Date: 9 March, 1995 (* 0.2 μ , ** 0.4 μ filter)

F	0 *	8	28	28	8	57	8	2	18	157	
		8	36	36	10	56	8	5	17	176	167
	0 **	8	20	34	9	48	7	3	17	146	
G		6	13	32	13	31	6	0	9	110	128
	0 *	7	15	30	7	41	5	0	9	114	
		3	13	34	7	48	7	0	13	125	120
H	0 **	4	10	27	5	49	5	0	6	106	
		3	8	16	3	37	3	0	5	75	91
	0 *	11	17	50	14	62	10	0	14	178	
I		10	14	43	19	60	9	0	14	169	174
	0 **	5	6	23	8	26	5	0	7	80	
		7	11	32	8	39	7	2	7	113	97
I	0 *	4	11	25	6	26	0	0	5	77	
		6	13	29	8	33	6	0	8	103	90
	0 **	9	11	51	22	46	0	4	14	157	
	8	9	41	12	32	0	0	16	118	138	

Appendix p.8

Stn.	Depth (m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	Average total concn. (nM)
------	--------------	-----	-----	-----	-----	-----	-----	-----	-----	-------	------------------------------

Cairns - Port Douglas

Date: 7-8 December, 1992

1	0	2	0	12	2	32	2	2	5	57	61		
		1	1	12	0	46	3	0	2	65			
	10	2	0	13	2	28	2	0	4	51		62	
		2	0	14	1	51	1	2	1	72			
	20	1	0	12	1	45	0	5	8	72		66	
		3	2	8	1	32	0	4	9	59			
	30	1	2	9	1	24	2	3	1	43		45	
		0	0	10	0	36	0	0	0	46			
	2	0	5	2	15	2	27	0	6	6		63	66
			2	2	18	1	40	0	3	2		68	
10		3	2	10	4	48	1	0	3	71	71		
		3	2	15	2	42	2	3	1	70			
25		2	5	17	1	35	0	4	2	66	74		
3	0	13	10	13	5	48	3	7	8	107	105		
		8	15	20	5	28	14	2	10	102			
	9	7	2	9	8	31	7	2	3	69		70	
		11	2	11	2	36	5	2	2	71			
4	0	5	7	19	4	55	10	3	5	108	102		
		12	4	16	6	41	9	4	4	96			
	4	12	9	28	25	50	4	0	0	128		106	
		4	5	16	8	36	7	0	8	84			
5	0	0	0	26	5	46	9	2	2	90	75		
		0	0	7	0	37	5	5	6	60			
	10	3	0	7	1	21	3	8	5	48		54	
		2	2	11	2	33	0	3	7	60			
	25	11	4	13	2	34	3	5	0	72		78	
6	0	2	2	10	4	32	2	4	2	58	60		
		5	0	9	0	45	0	2	0	61			
	10	8	2	15	2	26	0	0	1	54		60	
		9	0	12	2	39	0	0	4	66			
	25	2	1	14	0	30	0	4	5	56		55	
7	0	3	6	16	2	21	0	0	3	51	57		
		1	2	17	2	33	0	3	5	63			
	10	9	0	10	0	28	0	7	4	58		60	
		11	6	10	2	25	0	5	2	61			
	19	13	2	28	12	55	5	5	12	132		137	
8	0	4	5	14	3	23	2	3	3	57	60		
		6	6	11	0	27	2	3	8	63			
	10	6	4	13	0	39	3	4	2	71		78	
		2	5	14	3	50	3	2	6	85			

Appendix p.9

Stn.	Depth (m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	Average total concn. (nM)
Cairns - Port Douglas											
<u>Date: 7-10 January, 1993</u>											
1	0	1	1	27	1	76	8	1	7	122	
		3	1	23	1	47	2	3	2	82	102
	10	4	3	18	1	48	3	2	3	82	
		10	1	16	1	63	2	0	4	97	90
	20	5	7	10	2	57	1	0	2	84	
		16	18	24	3	51	3	0	1	116	100
2	30	9	12	21	8	71	0	0	3	124	
		10	5	13	2	79	5	0	5	119	122
	0	6	8	15	2	50	3	4	4	92	
		6	7	17	3	75	9	0	4	121	107
	10	1	0	24	0	68	2	3	2	100	
		4	2	15	1	63	1	2	3	91	96
3	20	2	0	11	0	50	5	5	1	74	
		1	1	10	2	68	1	1	5	89	82
	30	9	14	16	3	69	1	0	5	117	
		7	12	10	0	47	0	1	7	84	101
	0	10	21	22	9	61	2	1	6	132	
		12	16	25	9	58	0	6	5	131	132
4	10	6	16	18	5	45	7	0	10	107	
		7	10	11	4	71	2	2	16	123	115
	0	4	16	41	6	80	3	4	9	163	
		2	1	31	3	75	3	3	11	129	146
	5	14	11	43	11	98	11	6	6	200	
		5	10	37	7	106	6	4	7	182	<u>191</u>
5	0	2	1	22	1	46	2	2	2	78	
		4	1	25	1	43	3	5	4	86	82
	10	2	0	19	1	55	5	1	1	84	
		3	2	16	0	66	4	3	3	97	91
	20	0	1	18	1	53	8	2	2	85	
		2	2	25	0	47	2	3	4	85	85
6	30	2	7	14	1	56	0	2	1	83	
		4	3	17	0	65	1	3	3	96	90
	0	2	1	22	1	38	0	2	5	71	
		6	4	21	1	62	1	1	1	97	84
	10	1	2	25	1	58	2	2	2	93	
		3	1	26	1	72	3	1	0	107	100
8	25	4	0	21	0	51	5	1	4	86	
		1	1	20	2	48	1	1	3	77	82
	0	2	2	23	1	52	1	0	2	83	
		4	3	14	0	48	1	2	5	77	<u>80</u>
	10	2	1	21	0	59	2	0	1	86	
		3	4	15	1	47	6	3	2	81	84

Stn.	Depth (m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	Average total concn. (nM)
------	--------------	-----	-----	-----	-----	-----	-----	-----	-----	-------	------------------------------

Cairns - Port Douglas

Date: 22-23 February, 1994

1	0	5	3	19	4	48	4	3	7	93	
		4	4	22	5	47	4	8	7	101	97
	10	2	2	10	0	36	0	5	0	55	
		2	2	4	0	39	0	6	0	53	54
	20	2	1	10	0	37	0	7	0	57	
		1	2	15	0	41	0	7	0	66	62
	30	1	1	16	0	38	0	7	0	63	
		1	2	11	0	40	0	8	0	62	63
2	0	2	2	12	0	37	0	0	0	53	
		2	2	15	0	35	0	0	0	54	54
	10	2	1	13	0	30	0	0	0	46	
		1	1	13	0	27	0	0	0	42	44
	20	1	2	13	0	39	0	0	0	55	
		2	3	17	0	44	2	0	0	68	62
	30	2	2	17	2	41	0	0	0	64	
		2	2	14	0	32	0	0	0	50	57
3	0	4	1	11	0	28	0	0	0	44	
		1	2	11	1	33	0	0	0	48	46
	10	6	3	26	4	40	4	0	0	83	
		6	3	26	6	38	6	0	0	85	84
4	0	6	3	34	9	49	7	0	9	117	
		5	3	21	3	53	3	0	0	88	103
	5	4	3	20	0	45	5	0	0	77	
		3	2	20	0	33	0	0	0	58	68
6	0	2	3	20	3	34	3	0	0	65	
		2	3	16	0	39	3	0	0	63	64
	10	3	2	23	2	54	2	0	1	87	
		4	2	25	3	55	3	0	0	92	90
	20	3	3	27	4	55	2	0	11	105	
		2	2	21	0	53	1	7	0	86	96
	30	2	2	23	3	55	2	0	0	87	
		2	3	24	0	53	2	0	0	84	86
7	0	2	2	21	0	42	2	3	0	72	
		3	2	22	0	36	5	0	3	71	72
	10	2	3	23	0	41	2	4	0	75	
		4	2	23	0	36	0	3	0	68	72
	20	5	3	22	0	34	0	5	0	69	
		1	2	19	0	38	1	5	6	72	71
8	0	2	2	22	0	44	1	7	3	81	
		3	2	21	1	51	0	9	0	87	84
	10	2	2	19	0	55	0	9	0	87	
		2	2	17	0	36	1	9	13	80	84

Appendix p.11

Stn.	Depth (m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	Average total concn. (nM)
Cairns - Port Douglas											
Date: 28 February, 1995											
7	0	14	9	74	20	90	21	0	33	261	
		15	10	78	25	93	21	2	32	276	<u>269</u>
	10	8	6	36	13	68	11	0	15	157	
		5	5	23	7	48	7	9	12	116	137
	20	x	x	x	x	x	x	x	x	x	
8	0	8	8	32	11	50	6	0	14	129	129
		5	9	21	7	44	5	4	4	99	
	6	9	28	9	53	8	4	9	126	113	
		x	x	x	x	x	x	x	x	x	
	8	9	7	47	13	67	13	0	20	176	176
9	0	6	6	33	7	52	7	0	10	121	
		8	14	37	14	51	10	2	18	154	138
	10	3	9	11	5	25	2	0	7	62	
		5	5	22	5	38	4	0	7	86	74
	20	4	5	18	3	34	3	0	4	71	
10	0	3	5	15	3	33	3	0	4	66	69
		9	6	39	14	62	11	0	0	141	
	4	4	19	9	50	6	6	11	109	125	
		5	9	21	10	34	5	0	10	94	
	10	5	5	21	9	31	5	0	10	86	90
11	0	4	5	16	6	31	5	0	7	74	
		5	8	20	8	33	5	0	7	86	80
	3	3	3	12	3	35	5	0	6	67	
		3	4	13	4	38	5	0	8	75	71
	10	x	x	x	x	x	x	x	x	x	
12	0	11	15	44	18	66	14	16	23	207	207
		3	5	13	5	40	4	0	6	76	
	5	5	5	22	7	43	6	0	9	97	87
		6	6	27	10	49	14	0	0	112	
	30	5	6	22	7	41	6	0	5	92	102
13	0	4	6	9	2	15	5	5	9	55	
		5	8	13	5	21	7	1	4	64	60
	10	7	10	18	7	26	9	9	14	100	
		7	9	10	5	17	6	2	9	65	83
	20	3	4	13	2	37	4	0	5	68	
14	0	5	6	20	12	43	7	0	12	105	87
		3	7	8	4	21	3	0	5	51	
	4	8	8	5	14	2	0	8	49	<u>50</u>	
		5	7	9	4	17	3	1	6	52	
	8	4	8	9	2	25	5	4	8	65	59

Appendix p.12

Stn.	Depth (m)	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Total	Average total concn. (nM)
Daintree River											
<u>Date: 28 February, 1995</u>											
14	0	8	3	20	5	31	6	0	8	81	
		3	3	19	5	28	5	0	10	73	<u>77</u>
15	0	9	5	49	11	68	13	2	16	173	
		5	5	28	5	76	8	0	2	129	151
16	0	8	15	34	9	65	8	1	13	153	
		6	12	21	5	32	5	0	2	83	118
17	0	10	19	36	8	45	11	15	18	162	
		13	26	50	11	78	13	15	26	232	<u>197</u>
18	0	6	12	27	7	37	9	3	14	115	
		7	12	27	7	51	7	5	15	131	123