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VOLUME 41

Series Editors

A J Southward, P A Tyler, C M Young and L A Fuiman

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Interactions between Phytoplankton and Trace Metals in the Ocean

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This review assesses the degree to which phytoplankton in the contemporary oceans interact with the essential trace metals in their chemical environment as exemplified by the cycling of iron, manganese, cobalt, nickel and zinc. The toxic element cadmium is also considered because of the extent to which it is taken up. The stage is set by a brief consideration of the overall geochemical controls on the composition of sea water and their implications for the milieu within which life evolved.

The utilization of the elements within the cells is addressed with the consequent implications for optimizing the uptake of essential elements and controlling the ingress of potentially toxic elements. The impact of the change from an anoxic to an oxygenated atmosphere some 2 billion years ago on the availability of the basic building blocks for living systems is considered. The essential elements have to be delivered to the centres of synthesis in the appropriate ratios. The optimal tailoring of the flows of material into and out of the cell to meet the requirements for maintenance, growth and reproduction requires a carefully regulated internal economy. This internal economy sets the guidelines for the interaction of phytoplankton with the chemistry of their aqueous environment. The uptake mechanisms are explored using data derived from culture experiments indicating a significant degree of biological influence on the chemistry of the essential elements close to the cell surface (the near-field chemistry).

The perspective is then widened to consider the implications of these processes for the relative concentrations of the elements and their distribution throughout the world's oceans (the far-field chemistry). Case histories are followed for iron, manganese, copper, zinc, cobalt and nickel reviewing (a) their distribution in the oceans, (b) their biological availability, (c) their uptake and impact upon primary production. This external economy is intimately related to the feedback between the organisms and their environment.

The extent of recycling within the ocean system by the mutually dependent processes of photosynthesis and respiration provides a clear measure of the regulatory power of the biological system itself. This is analysed in the context of the Gaia hypothesis. Although the biological processes, to a large degree, control the availability and distribution of the essential trace metals in the oceans, the system does not appear to be optimized. For example cadmium, generally considered to be a non-essential element, is recycled more vigorously than any other element. Zinc in contrast appears to be rendered less accessible as the result of biological activity, and phytoplankton cells in the open ocean are straining at the limit of diffusive transport to obtain sufficient supplies.

1. INTRODUCTION

The intimate interactions between living things and their environment are dramatically illustrated by Alfred J. Lotka's neat re-casting of Shakespeare's imagery (Lotka, 1924).

> For the drama of life is like a puppet show in which stage, scenery, actors and all are made of the same stuff. The players, indeed, "have their exits and their entrances", but the exit is by way of translation into the substance of the stage; and each entrance is a transformation scene. So stage and players are all bound together in the close partnership of an intimate comedy; and if we would catch the spirit of the piece our attention must not all be absorbed on the characters alone, but must be extended also to the scene, of which they are born, on which they play their part, and with which, in a little while, they merge again.

These relationships are still a matter of considerable controversy 75 years after Lotka's perceptive insight. The interactions between micro-organisms and their environment have been addressed most creatively by the "Gaia hypothesis" (Lovelock, 1979; Bunyard and Goldsmith, 1988; Ehrlich, 1991). This hypothesis suggests that life is not simply a set of competing species reacting to change in a potentially hostile and uncontrollable environment. It considers that life is capable of influencing the environment on a global scale. Furthermore, it assumes that, through the process of natural selection, life (particularly microbial life) has evolved feedback mechanisms that help to regulate the environment so that it is conducive to life, both through the stabilization of environmental conditions and through the acceleration of change once the external forcing functions make a particular situation untenable. According to the Gaia hypothesis, an ability to modify the environment to suit the requirements of living things is just as valuable an attribute as the ability of an organism to adapt its own characteristics to take advantage of the changing environment. From this perspective, life and the environment are viewed more as a living tapestry than as a mosaic, with intimate links between the various components and with an overall structure that it is difficult to unpick.

The Gaia hypothesis has been clearly expounded (Lovelock, 1991, 1995; Westbroek, 1991) and thoroughly criticized (Kirchner 1989, 1991; Schneider and Boston, 1991; Robertson and Robinson, 1998). Its relationship to the theory of evolution has been eloquently explained (Lenton, 1998a; Lenton and Lovelock, 2000), and testable hypotheses have been posited and investigated (Lovelock *et al.*, 1972; Ferek *et al.*, 1986; Watts *et al.*, 1987; Ayers *et al.*, 1991; Liss *et al.*, 1993; Lovelock and Kump, 1994). These studies suggest that organisms have become so intimately involved

in the cycling of the elements in the atmosphere and in the oceans that, through a complex series of feedback mechanisms, there is regulation of the composition of the fluid environment. This regulation has been robust enough to counter the detrimental effects of external variations through negative feedback and to speed recovery from extinction events through positive feedback.

It is becoming increasingly clear that the interactions between marine phytoplankton and their environment have global implications in both directions (Barlow and Volk, 1990; Volk, 1997). Phytoplankton are not only strongly influenced by the chemical make-up of their environment, but they also influence directly the distribution and availability of the key essential elements upon which they depend. This evidence will be reviewed here.

2. THE MARINE CONTEXT

2.1. The seawater recipe

Life has existed on the earth for 3.8 billion years (Schidlowski *et al.*, 1979) and possibly longer (Mojzsis *et al.*, 1996; Nisbet and Fowler, 1996). The photosynthetic lifestyle was well established some 3.5 billion years ago (Nisbet and Fowler, 1996) when the atmosphere was still devoid of oxygen. The ocean would have covered the Earth's surface until the continents came into existence somewhere between 2.5 and 3 billion years ago. Even then, the relatively thin continental plates would have only slight elevation and shallow seas would have covered much of the globe's surface. The process of plate tectonics has been active for at least the last 1 billion years with the earliest recorded supercontinent (Rodinia) being identified 700 million years ago. The current episode of continental drift began with the break up of the supercontinent Pangea some 255 million years ago.

For nearly 2 billion years the world ocean was populated by complex microbial ecosystems (Margulis *et al.*, 1986; Margulis, 1991; Nisbet, 1995) driving and interlinking the chemical cycles of the essential elements (Margulis and Sagan, 1986). The simple, non-nucleated cells (prokaryotes) working in concert were able to engineer major changes in the earth's environment. Most notably, from the present perspective, photosynthesizing bacteria liberated oxygen as a "toxic" by-product, yielding 1% of oxygen by volume in the atmosphere by 2 billion years ago (Holland, 1984, 1990, 1994). By 1.5 billion years ago, the levels of free oxygen in the atmosphere had reached the point where novel organisms with nucleated cells (eukaryotes) could evolve to take advantage of this new hydrogen acceptor (Margulis and Sagan, 1986; Margulis, 1991). Lovelock (1979, p. 69) nicely encapsulates the impact of this transition:

> The present level of oxygen tension is to the contemporary biosphere what the high voltage electricity supply is to our twentieth-century way of life. Things can go on without it, but the potentialities are substantially reduced.

The mystery of the apparent stagnation of evolution at the unicellular stage for 80% of life's history has raised considerable debate (Maddox, 1994). There are some interesting theories related to (a) the lowering of the alkalinity of the oceans (Kempe and Kazmierczak, 1994), (b) the attainment of an appropriately low global mean temperature (Schwartzman and Volk, 1991; Schwartzman *et al.*, 1995; Schwartzman and Shore, 1996), (c) the maintenance of a critical level of dissolved oxygen in the sea (Smith and Szathmáry, 1995, 1999), (d) the establishment of the required cocktail of available trace metals (Williams, 1981), or (e) the existence of oceanic lacunae in a snowball earth (Hyde *et al.*, 2000; Runnegar, 2000). Whatever the cause, it is only in the last 600 million years or so that eukaryotic cells have linked symbiotically to evolve multicellular life forms (metazoa) (Margulis and Fester, 1991). The metazoan zooplankton play a crucial role in the recycling of the essential elements in the contemporary ocean by generating a flux of particulate organic matter and mineral phases from the euphotic zone into the deep ocean.

Although the deep hot biosphere (Gold, 1992, 1998) and hydrothermal vents (Nisbet, 1995; Nisbet and Fowler, 1996; Russell and Hall, 1997) may have played a part, the oceans are rightly considered to be the cradle of life, providing the prime environment during the formative millennia when the basic ground rules for cellular metabolism were established. An aqueous environment provides an excellent milieu for the transport of nutrient elements to growing cells and for the removal of waste products, which then become accessible to other organisms as a resource. It also provides a good measure of protection against UV radiation.

2.1.1. *Concentrations of the elements in sea water*

All of the elements are present in dissolved form in sea water (Appendix I). Their global mean concentrations range over 12 orders of magnitude. There is strong evidence that underlying geochemical mechanisms, modulated by the periodic chemical properties of the elements, constrain the concentrations of the elements in sea water and crudely define their relative concentrations (Turner and Whitfield, 1979a; Whitfield and Turner, 1979, 1987; Turner *et al.*, 1980; Li, 1981, 1991; Kumar, 1983; Green and Chave, 1988). The crustal abundance of the elements (dictated by cosmochemistry and volcanic fractionation) defines the composition of the suspended sediment in the rivers (Martin and Meybeck, 1979; Martin and Whitfield, 1983) and of the dust in the atmosphere (Duce *et al.*, 1991),

which are transported from the land to the ocean. The composition of the dissolved input from the rivers (Martin and Meybeck, 1979; Martin and Whitfield, 1983) depends upon the inorganic chemical processes that control the uptake and release of the elements from the particle surfaces (Whitfield and Turner, 1979). These materials interact within the oceans with the acid volatile materials released from vulcanism along the mid-oceanic ridges (Elderfield and Schultz, 1996). The composition of sea water is further modified by exchanges with the slowly sinking particles.

These processes have been active throughout the Earth's history and it is likely that the crustal material will have cycled through this system 10 times or more since the Earth was formed (MacKenzie and Wollast, 1977). Simple and significant correlations have resulted between the concentrations of the elements in contemporary sea water and the periodic, inorganic properties of the elements. The parameters that define the intensity of interaction between the dissolved elements and particles within the ocean have been elegantly reviewed (Li, 1991). The details of these correlations have been discussed extensively (Turner and Whitfield, 1979a, 1983; Whitfield and Turner, 1979, 1987; Turner *et al.*, 1980; Li, 1981, 1982, 1991; Whitfield, 1981, 1982). The interactions represented are capable of defining the absolute and relative concentrations of the elements in sea water only to within a factor of a hundred or so – a rather coarse regulation to ensure the continuity of the evolution of life (Morel and Hudson, 1985). Against this broadly defined backdrop, the detailed biological and geochemical processes at work within the oceans have defined the composition that we observe today (Appendix I).

2.1.2. *Chemical form and biological availability*

The same inorganic properties that provide a broad rationalization for the composition of sea water also provide useful guidelines for defining the chemical form, or speciation of the elements in sea water (Turner *et al.*, 1981). The chemical speciation of the elements in a medium as complex as sea water can have a controlling influence on their biological availability; for essential trace metals, it is usually the free metal ion that is most readily assimilated. Equally important are the correlations that can be used to define the strength of the hydrolysis of the elements in sea water (Turner and Whitfield, 1983; Turner *et al.*, 1981; Whitfield and Turner, 1983, 1987; Clegg and Sarmiento, 1989). These identify the elements that are likely to be most aggressively scavenged from the water column by particles. The underlying inorganic chemical correlations also influence the selection of the elements to perform essential functions in living cells (da Silva and Williams, 1991; Williams and da Silva, 1996).

A simple way of demonstrating such relationships is to plot a function ($\Delta\beta$) representing the ability of the element in question to form covalent bonds:

$$\Delta\beta = \log \beta^{o}_{MF} - \log \beta^{o}_{MCl}$$

(where β^{o} is the stoichiometric formation constant of the complex) against a function (z^2/r, where z is the ionic charge and r the ionic radius) representing its ability to form electrostatic bonds. The resulting diagram (Turner *et al.*, 1981; Turner and Whitfield, 1983), termed the "complexation field" diagram, clearly groups together the elements that exhibit similar chemistries within the ocean system (Figure 1).

2.1.3. *Vertical concentration profiles*

The elements likely to be strongly adsorbed onto the surfaces of small particles (Figure 1, the scavenged elements) will be those that are hydrolysed under the ambient conditions in sea water (Turner *et al.*, 1981). These elements can be identified primarily by their large z^2/r values and are therefore grouped together on the complexation field diagram (Turner and Whitfield, 1983) (Figure 1, Appendix I). Scavenging indices have been devised to estimate the extent of uptake and transport of hydrolysed trace metals via this route (Whitfield and Turner, 1987; Clegg and Sarmiento, 1989). Elements with insoluble higher oxidation states (e.g. Co, Ce, Pb) are also prone to oxidative scavenging onto the surfaces of iron or manganese oxyhydroxide particles. This can occur even if the lower oxidation state is thermodynamically stable in oxygenated sea water.

The scavenged elements will be stripped from the water column at all depths as the small particles are aggregated and disaggregated, and adsorbed material is progressively transported downwards on the large particles. The process will be significant even on colloidal particles ($< 0.1\ \mu$m). The vertical profiles of these elements are characterized by a decline in concentration with depth. Lead provides a classic example of this effect (Flegal and Patterson, 1983), as its primary source is via aeolian input (most of it now from the degradation of alkyl leads in petroleum). Scavenged elements have short mean oceanic residence times[1] (<1000 yr) and low concentrations in sea water (typically 10^{-14} M to 10^{-11} M).

At the other end of the particulate uptake spectrum are the elements with small values of z^2/r, which accumulate in the oceans and have long

[1]Mean oceanic residence time T_Y = (total mass of Y in oceans)/(rate of addition or removal of Y).

Figure 1 The complexation field diagram. The function z^2/r is plotted on the ordinate. This represents the strength of electrostatic interaction exerted by the cation and is a measure of Lewis acid strength (ability to accept a pair of electrons). The function $\Delta\beta$ is plotted on the abscissa (see text). This represents the strength of covalent interactions exerted by the cation. Elements in the white zone are present as the free cations and inorganic complexes in sea water. Elements in the light grey zone experience strong hydrolytic scavenging. Elements in the dark grey zone are present as condensed oxyanions or oxocations. Essential elements are set in boxes. Recycled elements are shown in italic text. Accumulated elements are encircled. Scavenged elements are shown in plain text. (For detailed analysis see Turner *et al.*, 1981; Turner and Whitfield, 1983.)

mean oceanic residence times ($>10^5$ yr) and relatively high concentrations (10^{-9} M to 10^{-1} M, depending on crustal abundance). These elements show no fractionation between the surface and the deep water (except that resulting from the addition and removal of water) and they have constant concentration ratios to one another. These conservative (or accumulated) elements are also grouped together on the complexation field diagram (Figure 1).

Elements that are taken up actively by phytoplankton, or which are taken up by analogy, will exhibit vertical profiles with low concentrations in the surface layer but enhanced concentrations in the deeper water as the

larger particles are broken down. These recycled elements are characterized by intermediate residence times (10^3 to 10^5 yr) and concentrations (10^{-12} to 10^{-5} M), and they form a coherent group in the complexation field diagram.

A number of other factors influence the nature of the vertical concentration profiles of the elements. As deep water ages in its transit from the North Atlantic to the North Pacific (a time span of some 1500 yr) it becomes progressively more depleted in elements that are adsorbed onto the particles (scavenged elements) and it accumulates elements released from the decaying particulate matter (the recycled elements). The water also becomes oxygen-poor as it fuels the respiratory cycles and more acid as it accumulates the CO_2 released.

The deep water does not just carry the history of particle production in the surface water that are its geographical companions at the time of sampling. It brings with it preformed nutrients as well as the consequences of mixing in the deep ocean between adjacent waters masses and between the sediment and the water column (Saagar, 1994). Lateral transport from the shelf and shelf slope regions can also carry with it signals from the release of elements from anoxic, organic-rich sediments at intermediate depths. This feature is particularly noticeable off the western seaboard of North America and in the northern Indian Ocean.

The present configuration of the continents also ensures that the Atlantic Ocean is relatively more affected by inputs from land than the Pacific Ocean. It therefore receives a greater flux of windborne dust and atmospheric contaminants than the Pacific, and this can penetrate even the regions most remote from land.

3. THE INTERNAL ECONOMY FOR ESSENTIAL TRACE METALS

3.1. The establishment of the internal economy

There are four conditions that must be met, in sequence, for an element to perform an effective role within cell mechanisms (Williams, 1981).

1. It must be sufficiently abundant to provide a reliable resource.
2. Its chemistry in solution must allow it to be readily available for uptake.
3. It must be capable of being caught and held by the cell in a suitable "kinetic trap" from which it can be transferred for use within the cell.
4. It must be capable of performing a useful function within the cell in cooperation with the other ingredients.

The differences between prokaryotic and eukaryotic cells (Table 1) have particular significance when considering the workings of the internal economy of these cells and their interactions with sea water.

The evolution of eukaryotic cells is considered to be the consequence of the symbiotic aggregation of primitive prokaryotic cells (Margulis and Fester, 1991; Sapp, 1994; Margulis, 1998). Recent genetic studies suggest that the Archaebacteria were the ancestral cells from which the eukaryotes evolved (Doolittle, 1998, 1999). The entrapment of prokaryotic metabolic requirements within the newly emerging nucleated cells stamped a certain conservatism on the requirements of these cells for the essential elements. The more ancient lineage of the prokaryotic cells is still reflected in their responses to trace elements in the oceans.

From the ninety or so natural elements in the periodic table, life has selected about two dozen elements to perform the essential structural and metabolic functions within prokaryotic and eukaryotic cells (Williams and da Silva, 1996). Even the simplest single cells require a minimum of 12 elements to perform their basic functions (Table 2, categories 1–3 plus Fe).

Table 1 Characteristics of prokaryotic and eukaryotic phytoplankton[a]

Small cell prokaryotes (notably cyanobacteria, prochlorophytes)	Large cell eukaryotes (notably diatoms)
Have large surface:volume ratios and can therefore assimilate nutrients, etc. efficiently	Have smaller surface:volume ratios and can therefore assimilate nutrients, etc. less efficiently
Have low total requirements per cell for nutrient elements and can therefore reach their reproductive (division) requirements quickly	Have high total requirements per cell for nutrient elements and therefore reach their reproductive (division) requirements slowly
Divide rapidly (hours)	Divide slowly (days)
Have high non-chlorophyll pigment (NPC):chlorophyll *a* ratios and are efficient at absorbing incident radiation	Have low non-chlorophyll pigment (NPC):chlorophyll *a* ratios and are relatively inefficient at absorbing incident radiation
Function most effectively in nutrient-poor conditions	Function most effectively in nutrient-rich conditions
Are grazed by small protozoa which have a comparable multiplication time	Are grazed by large copepods, which have a long multiplication time and can therefore not graze down a bloom in its early stages (but NB salps have evolved to deal with this situation)

[a] This is a caricature based upon the most common prokaryotic and eukaryotic phytoplankton. Overall there is a continuum in sizes and in division rates.

The essential elements are grouped primarily in the wings of the periodic table and in the first row of the transition series, generally with atomic numbers <35 (Figure 2). Selection is first dictated by the abundance of the elements in sea water as prescribed by the overall geochemical controls. Indeed the distribution of the essential elements in the periodic table falls

Table 2 Main biochemical functions of the chemical elements.[a]

General function	Elements	Chemical form	Examples of specific functions
(1) Structural functions (biological compounds)	H, O, C, N, P, S	Combined in organic compounds	Components of biological molecules; formation of tissues, membranes, internal structures, energy storage, etc.
(2) Electrochemical functions	H, Na, K, Cl (Mg) (Ca), HPO_4^{2-}	As free ions	Osmotic regulation; transmission of messages; production of metabolic energy
(3) Mechanical effects	Ca (Mg), HPO_4^{2-}	As free ions exchanging with bound ions	Lysis of vesicles
(4) Acid-base catalysis	Zn (Ni)	Combined in enzymes	Food digestion (Zn); hydrolysis of urea (Ni)
(5) Redox catalysis	Fe, Cu, Mn, Mo, Si (Co) (Ni) (V)	Combined in enzymes	Reactions with oxygen (Fe, Cu); oxygen evolution (Mn); nitrogen fixation (Mo, Fe); inhibition of lipid peroxidation (Se); reduction of nucleotides (Co); reactions with H_2 (Ni); bromoperoxidase activity (V)
(6) Various specific functions	Mg	In chlorophyll	Light harvesting in photosynthesis
	Fe, Cu	In proteins	Transport of oxygen
	Fe, Ca, Si, Ba, Sr	Combined in sparingly soluble inorganic compounds	Skeletons, shells, protection, buoyancy adjustment

[a] Adapted with the permission of the copyright holders Oxford University Press from: da Silva, F. J. J. R. and Williams, R. J. P. (1991). "The Biological Chemistry of the Elements". Clarendon Press, Oxford, page 143.

Figure 2 Periodic table showing the relationship between the biological requirement and the concentration of the elements i the contemporary oceans. Fe, Co and W are micronutrients that are present at concentrations $<10^{-9}$ M.

Table 3 Possible differences in the availability of elements between the Archaean ocean and the modern ocean.[a]

Element	Archaean ocean	Modern ocean	Concentration shift[b]
As	As_2S_3 (s)	AsO_4^{3-}	+
I	I_2, I^-	IO_3^-, I^-	+
N	NH_4^+	NO_3^-	−−
S	HS^-, S^{2-}	SO_4^{2-}	+
Se	HSe^-, Se^{2-}	SeO_3^-, SeO_4^-	+
Cd	CdS (s)	Cd^{2+}	++
Co	Co^{2+}	Co^{3+}	−
Cu	CuS (s), Cu_2S (s)	Cu^{2+}	+10
Cr	Cr_2S_3 (s), $Cr(OH)_3$ (s)	Cr (VI), *Cr (III)*	+
Fe	Fe (II) 1 mM	Fe^{3+} $<10^{-17}$ M *Fe (II)*	−14
Mn	Mn (II)	Mn (IV) (s) *Mn (II)*	−2
Mo	MoS_3 (s), MoS_4^{2-} Mo (VI)	Mo (VI)	+1
Ni	Ni^{2+}	Ni^{2+}	?
V	V (III), V (IV) sulphides	VO_4^{3-}	+
W	WS_4^{2-}	WO_4^{2-}	
Zn	ZnS (s), Zn^{2+}	Zn^{2+}	+2 to 4

[a] Compiled from data in Williams and da Silva, 1966; da Silva and Williams, 1991; Dyrssen and Kremling, 1990.
[b] Archaean to modern: + increase, − decrease, ? uncertain. Numbers indicate the approximate power of 10 shift in concentration (Williams and da Silva, 1996). Italics indicate a metastable form, (s) indicates a solid phase.

almost entirely within the range of elements with total concentrations $>10^{-9}$ M in the contemporary ocean (Figure 2, Appendix I) (da Silva and Williams, 1991; Williams and da Silva, 1996).

The second requirement for selection is availability, and the roots of the selection process lie some 4 billion years ago when the prokaryotes evolved in an anoxic Archaean Ocean. This was a reducing environment, probably rich in hydrogen sulphide, and the forms of some of the key trace elements would be quite different to those encountered in the present well-oxygenated ocean (Table 3). Concentrations of Fe, Co and Mn were probably much higher then because these elements would be present in their reduced divalent forms and the concentrations of Cu, Zn, Cd and Pb would have been much lower because of their tendencies to form insoluble sulphides. Elements such as Se, Mo and U would also be held largely in the solid phase. Nitrogen would have been present primarily as

the ammonium ion (NH_4^+) or molecular nitrogen. These tendencies are apparent today in anoxic sediments and in the stagnant waters found in enclosed basins and in the deep ocean trenches (Dyrssen and Wedborg, 1975; Jacobs *et al.*, 1987; Byrne *et al.*, 1988; Dyrssen, 1988; Haraldsson and Westerlund, 1988; German and Elderfield, 1990).

A significant shift in seawater composition would accompany the long transition to an oxygen-containing atmosphere around two billion years ago. The evolution of the eukaryotes (Margulis and Sagan, 1986; Knoll and Bauld, 1989; Knoll, 1994; Falkowski and Raven, 1997; Lee, 1999) would therefore have taken place in an ocean becoming progressively deficient in Fe, Mn and Co, where nitrogen was present primarily as nitrate ions (NO_3^-), and where relatively high concentrations of potentially toxic elements such as Cu, Cd and Pb were encountered. These changes make their presence felt when we consider the evolution of the functions of the elements within the cells that encountered these new conditions. However, the oxygenated microenvironments produced by some photosynthetic bacteria would have subjected them, and those organisms living in their close proximity, to these additional selective pressures long before free oxygen became ubiquitous.

The criterion of availability in the hierarchy of necessary conditions for the biological utilization of the elements is a plastic one because the species that survived the oxygen transgression, or evolved when oxygen levels were high, would still have inbuilt requirements for key elements such as iron and manganese from their ancient origins despite the drastic reduction in their availability. Uptake mechanisms would therefore need to be honed, at some energetic cost, to ensure the most efficient collection of those key elements that became less readily available, but for which there were no substitutes. Since the rise of oxygen levels, reducing conditions have been maintained within prokaryotic and eukaryotic cells despite the oxidizing exterior, but also at significant metabolic cost. Within the reducing conditions of the cytoplasm, Mn and more importantly Fe continued to perform key functions at the centre of the cells' metabolism.

The elements required for the basic structural, electrical and mechanical functions (the macronutrients, categories 1–3, Table 2; Figure 3) are common to all prokaryotic and eukaryotic cells. It is probably no coincidence that their overall concentrations and availability changed little during the transition to an oxygenated atmosphere. The case of sulphur is interesting because the total concentration of sulphur probably changed by less than an order of magnitude despite a major transition from HS^- to SO_4^{2-}.

Three elements (Zn, Cu, Cd) that were released into solution by the oxygenation of the oceans (Table 3) are present as free divalent cations

Figure 3 The links between the functions of the nutrient elements within the cell. This intimate linking of the functions of the elements provides the basis for internal homeostasis, which in turn imprints its needs on the external environment. The trace metals considered here are encompassed by the shaded boxes. (Redrawn from information in Williams and da Silva, 1996.)

in solution and so they are readily transported across the cell membranes of both prokaryotes and eukaryotes. They are potentially toxic because of their ability to bind to sulphur sites and to interfere with the acid-base (Zn, Cu) and redox (Cu) chemistry of the cell. They would initially threaten this conservative stronghold and would therefore have to be rendered harmless by complexation. Once they were under control, advantage could be taken of the presence of these elements and their unique chemistries to broaden the range of transformations possible within the cell and at its periphery.

3.2. The functions of the essential trace metals

The essential trace metals (micronutrients) are used for a variety of catalytic and electron transfer functions and their specific roles may be different between different groups of unicellular organisms. The variety of functions performed by these elements (Table 2, Table 4) in different groups of organisms can be ascribed at least in part to their gradual

Table 4 Involvement of trace metals in specific enzyme systems.[a]

Metal ion	Examples
Co	• Vitamin B_{12}:rearrangements, reduction, C and H transfer reactions with glycols and ribose
Cu	• Laccase, oxidases
	• Plastocyanin: photosynthetic electron transport
	• Cytochrome *c* oxidase: mitochondrial electron transport
Fe	• Cytochrome oxidase: reduction of oxygen to water
	• Cytochrome P-450: O-insertion from O_2, detoxification
	• Cytochromes *b* and *c*: electron transport in respiration and photosynthesis
	• Cytochrome *f*: photosynthetic electron transport
	• Ferredoxin: electron transport in photosynthesis and nitrogen fixation
	• Iron-sulphur proteins: electron transport in respiration and photosynthesis
	• Nitrate and nitrite reductases: reduction to ammonium
Fe (Se) (V) (Mn)	• Catalase, peroxidases in H_2O_2 breakdown and in reactions involving halogens
Fe, Mn, Cu	• Superoxide dismutases: disproportionation of O_2^- radicals to O_2 and H_2O_2
Fe, Mn	• Acid phosphatase
Mn	• Oxygen-generating system of photosynthesis
Mo	• Nitrate and nitrite reductases: reduction to ammonium
	• Sulphate reductase
Mo (Fe) (V)	• Nitrogenase: nitrogen fixation
Ni	• Urease: hydrolysis of urea
Ni (Fe)	• Methanogenesis
Zn (Cd)	• Carbonic anhydrase: hydration and dehydration of CO_2
Zn	• Alkaline phosphatase: hydrolysis of phosphate esters
	• Hormone control (peptidases)
	• DNA and RNA polymerases: nucleic acid replication and polymerization

[a] Compiled from summaries provided by da Silva and Williams (1991) and Sunda (1989).

assimilation into prokaryotic and eukaryotic metabolisms. Trace metals perform essential roles (Figure 3) in major element assimilation, biological energy capture, information transfer and control, and the development of shape. These elements are also used in combinations to achieve some essential transformations such as the role of Cu and Zn in cytochrome oxidases, and Fe and Mo in nitrogenases (Williams and da Silva, 1996). Relevant physicochemical properties of the elements are summarized in Table 5. This review will focus on the essential transition metals from Mn to Zn in the periodic table, as these are potentially limiting nutrients that form a coherent group in the complexation field diagram. The essential

Table 5 Physicochemical properties of essential trace metals.

	Dissolved metal in surface sea water				Free M^{2+} property			
Metal	Inorganic species[a]	$[M_T]^b$ $\times 10^{-9}$ M	$[M']^b$ $\times 10^{-9}$ M	pM^{2+}	ΔE^c	z^2/r (Å)	χ^d	$k_L{}^e$
Mn	Mn^{2+} $MnCl^+$	1	1	9.2	717	4.8	1.60	3×10^7
Fe	$Fe(OH)_4^-$ $Fe(OH)_2^+$	0.06	0.06	20.2	759	5.1	1.65	2×10^6
Co	Co^{2+}, $CoCl^+$ $CoCO_3$	0.01 0.02‡	0.01 0.02‡	10.9	760	5.3	1.7	4×10^6
Ni	Ni^{2+} $NiCl^+$ $NiCO_3$	2	2	9.0	737	5.8	1.75	1×10^5
Cu	$CuCO_3$ $CuOH^+$ Cu^{2+}	1 0.6‡	0.002 0.002‡	12.7	745	5.5	1.75	1×10^9
Zn	Zn^{2+} $ZnCl^+$ $ZnOH^+$	0.1 0.2‡	0.002 0.004‡	11.7	906	5.3	1.66	4×10^7
Cd	$CdCl_2$ $CdCl^+$ $CdCl_3^-$	0.001 0.002‡	0.001 0.0006‡	13.7	868	4.2	1.46	4×10^8

[a]Turner *et al.* (1981).
[b]Morel *et al.* (1991) with additional concentration data (‡) from the North Pacific (Hudson and Morel, 1993). $[M']$ represents the concentration of metal not complexed by organic ligands.
[c]First ionization energy ($M \rightarrow M^+$) kJ mol^{-1} (Emsley, 1989).
[d]Allred-Rochow electronegativity (Emsley, 1989).
[e]Ligand exchange rate constant (s^{-1}) = water exchange rate constant (k_{H_2O}) (Morel *et al.*, 1991).

elements molybdenum, vanadium and tungsten are present as oxyanions in sea water and molybdenum in particular is in plentiful supply. There is no evidence of their lack of availability limiting phytoplankton growth.

3.2.1. *Manganese*

Manganese is a Lewis acid with a useful redox chemistry involving three accessible oxidation states (II, III, IV) (da Silva and Williams, 1991). The complexes that Mn (II) forms with S- and N-ligands are stronger than those for Ca and Mg but weaker than for Fe, Cu and Zn. In complexing with N-ligands Mn is in competition with Zn (II) and Cu (II), which dominate (Irving-Williams series). With O-ligands it has similar binding strengths to Mg, which is present in much higher concentrations. As Mn (III) it might also perform a useful function catalysing electron transfers.

Mn was probably the most readily available transition element in the Archaean ocean because it does not form insoluble sulphides. Manganese is also more readily available than iron in the Mn (II) form in oxygenated systems since its oxidation to Mn (IV), although thermodynamically favoured, is very slow. There would therefore be a relatively small change in the availability of Mn (II) when the atmosphere was oxygenated.

Mn (III) and Fe (III) are so similar in binding and kinetics that they are used almost indiscriminately in superoxide dismutases, acid phosphatases, ribonucleotide reductases and in transfers across membranes. Manganese is almost certainly selected for some functions because of its greater availability, rather than its unique chemistry.

The internal concentrations of Mn in the cytoplasm of eukaryotes and prokaryotes are usually rather low ($<10^{-8}$ M). It is an active component of superoxide dismutase in prokaryotes. It is actively pumped out of the cytoplasm and contained in vesicles or organelles (mitochondria and chloroplasts) in eukaryotes. Mn is closely involved in the functions of these organelles and in glycolization within the Golgi apparatus in eukaryotic cells. It is essential for oxygen release from water in photosynthesis system II in chloroplasts (Falkowski and Raven, 1997). Four manganese atoms together are involved in the active site.

3.2.2. *Iron*

Iron can be persuaded to occupy three oxidation states within the cell (II, III, IV) (da Silva and Williams, 1991). Iron is fundamental to the physiology of prokaryotic and eukaryotic cells. It is required for photosynthesis and respiratory electron transport, for nitrate, nitrite and sulphate reduction and for nitrogen fixation. It also acts as an acid catalyst in hydrolytic enzymes. The archaebacteria are heavily dependent on iron, nickel and cobalt. In eubacteria, a dominant dependence on iron is most noticeable (da Silva and Williams, 1991). Iron is involved in oxidation-reduction catalysis, bioenergetics, acid-base reactions and control systems. Anaerobic prokaryotes can assimilate iron directly as Fe (II) or via Fe-S complexes. Iron is the most common metallic component of membranes (after the group IA and IIA cations) and is usually present as Fe/S proteins (da Silva and Williams, 1991). Within the cytoplasm, Fe_nS_n clusters form, which have an ancient origin. Hydrogenases that involve iron alone are derived from Fe_4S_4 clusters.

In the transition from an anoxic to an oxygenated environment, the levels of total dissolved ($<0.4\,\mu$m diameter) iron in the ocean would have fallen by about 12 orders of magnitude. The central importance of iron to cellular metabolism gave rise to new strategies for sequestering it

for use inside the cell, in competition against the removal of iron from solution by oxidation and hydrolysis.

Aerobic prokaryotes such as the cyanobacteria release strong, iron-specific complexing agents (siderophores) that can be transported through their cell membranes (da Silva and Williams, 1991). Within the cell, the iron is bound to ferric uptake regulatory (or FUR) proteins. It appears that zinc may also be necessary to help bind the FUR proteins to DNA (Williams and da Silva, 1996). One of the key players in the detoxification of reactive oxygen species within the cells is superoxide dismutase. Iron plays a central role here, although Cu and Zn are also used (Asada *et al.*, 1977; Raven *et al.*, 1999).

Within eukaryotes, much iron/sulphur chemistry is confined to the mitochondria and to the chloroplasts. Iron performs a central role in the photosynthetic process through its essential involvement in photosystems I and II. Non-haem iron is implicated in redox chemistry that involves free radicals. It is therefore usually carried out in compartments within the cell. Haem iron also plays a major role in coupled redox reactions in eukaryotes. The porphyrin traps the iron as Fe (II) so that it is not in equilibrium with free iron. However, the Fe is still accessible for electron transfer reactions. The haem proteins perform essential functions in electron transfer, storage and transport and oxidases and oxygenases.

3.2.3. *Cobalt and nickel*

These elements would be important in the electron-rich early environment where catalysts were needed for handling small molecules (CH_4, CO, H_2, H_2S) and they still perform these functions in anaerobic prokaryotes. Their roles have evolved little in eukaryotes.

The concentrations of cobalt in sea water as Co (II) are very low ($<10^{-10}$ M). There is evidence for the biological oxidation of Co (II) to Co (III) and for its removal by scavenging on particles (Lee and Fisher, 1993a). The redox functions of Ni and Co are replaceable by Fe, Cu or Mn in eukaryotes. Their acid-base chemistry can be replaced by Zn. The exchange kinetics of Ni are very slow compared with Zn, Cu and Mg as defined by the inner sphere H_2O substitution rate constants (k_{H_2O}, Table 5) for the divalent ions (Frey and Stuehr, 1974).

The roles of Co and Ni in eukaryotes therefore tend to be few and specialized. Nickel locked in vesicles is mainly involved in ureases for nitrogen acquisition. Cobalt has its main role in vitamin B_{12}. It is used in the transfer of methyl groups and rearrangement reactions that can now be carried out in eukaryotes by Fe- and Mn-containing enzymes using oxygen.

3.2.4. *Copper*

Copper has three accessible oxidation states (I, II, III) in biological systems, spanning the redox potential range +0.2 to +0.8 V. Cu (I) has a high electron affinity (it acts as a stronger Lewis acid than Zn) and is the most effective monovalent cation for binding organic ligands. It is the only monovalent cation that is a good Lewis acid and is available to biological systems. Cu (II) is the most effective divalent cation for binding organic ligands.

Copper concentrations would have increased dramatically (possibly as much as 10 orders of magnitude, Table 3) during the transition to an oxygenated atmosphere. The rise in copper availability may have occurred later (about 1.5 billion years ago) than the release of zinc (Williams and da Silva, 1996). Biological systems probably did not experience high copper concentrations before the arrival of oxygen, so that it will be a potent toxic element to prokaryotic organisms. Copper is found in the membrane or periplasmic space of denitrifying prokaryotes as a constituent of reductases of the lower oxides produced by the reduction of nitrate via Mo-containing enzymes.

Cu has a major role in photosynthesis as a structural and electron exchange component of plastocyanin. This plays a role in the transfer of electrons from photosystem II (PSII) to photosystem I (PSI) where they are used to reduce NADP to NADPH (da Silva and Williams, 1991; Falkowski and Raven, 1997). This function can also be performed by cytochrome c_6, which contains iron rather than copper. Since the availability of iron plummeted during the oxygenation of the oceans whereas the availability of copper rose dramatically, we would expect to observe a transition from the use of cytochrome c_6 to the use of plastocyanin upon moving from the cyanobacteria to the diatoms. In fact the opposite is the case (Raven *et al.*, 1999), suggesting a very early origin for the use of plastocyanin (before 2 billion years ago). From studies of molecular phylogeny, Cu-containing cytochrome oxidases that are used in respiration are also considered to have evolved at a similar time (Schäfer *et al.*, 1996). It is possible that this anomaly results from the evolution of these requirements in locally oxygenated micro-environments within the prevailing anoxic ocean. There is no difficulty in finding anoxic systems functioning perfectly adequately within millimetres of our present oxygenated ocean. This complicates the simple picture of a succession in the use of essential elements (da Silva and Williams, 1991).

In eukaryotes, the most common use of copper is in electron transfer (oxidative enzymes and energy capture). Copper is usually locked in vesicles or banished to the external regime of the cell membrane. It has become the major external redox element in eukaryotes (da Silva and

Williams, 1991), replacing iron as a component of cell surface oxidases (e.g. in the external metabolism of nitrogen oxides and in the cross-linking of extracellular matrices). The copper enzymes oxidize ascorbate, phenols, some amines, ferrous iron and some sugars. A notable exception to the external quarantine of copper is its use in superoxide dismutase for the detoxification of oxygen. Cytochrome oxidase has two copper atoms. This is the only part of the energy capture system of mitochondrial and bacterial membranes that depends upon copper.

3.2.5. *Zinc*

Zn has a high electron affinity (Lewis acid strength, see Table 5) and it has a highly localized charge (or small z^2/r value, Figure 1). It can bind to the same centres as iron but it does not exhibit any redox chemistry. These factors enable it to function as a useful catalyst centre. Since Zn (II) forms insoluble sulphide compounds, a significant rise in zinc concentrations (by four orders of magnitude, Table 3) may have accompanied the oxygenation of the ocean and atmosphere. It would therefore be expected to be more commonly used in eukaryotes than in prokaryotes.

Zn is a commonly used catalyst of hydrolysis and is employed in signalling systems. Zinc thiolate complexes have apparently replaced nickel-sulphur compounds in eukaryotes. Copper as Cu (I) can generally bind more strongly to sulphur-based complexes than zinc. However, the role of zinc as a structural element in the "zinc finger" thiolates (which control DNA expression) has been retained because of the relatively weak binding of cysteine to Cu (I) in the presence of the metallothionein compounds. Zinc is also important in structural cross-linking and in the organization of chromosomes. It is the most common catalytic metal ion in the cytoplasm. It is involved in digestive enzymes outside the cell or in vesicles but it is rarely in contact with the cell membrane. In phytoplankton, Zn has a particularly important role in carbonic anhydrase. This is one of the most catalytically active enzymes known and catalyses the exchange between gaseous CO_2 and carbonic acid, which is the rate-limiting step in the transport of molecular CO_2 to RubisCo, and therefore accelerates the reaction scheme:

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \qquad \text{(fast)}$$
$$H_2CO_3 \leftrightarrow CO_2 + H_2O \qquad \text{(slow)}$$

Da Silva and Williams (1991) suggest that Co (II) is generally the most effective substitute for Zn in enzymes, although Cd substitution

also occurs in eukaryotic phytoplankton (Cullen *et al.*, 1999). Overall zinc plays a major regulatory role in the metabolism of cells. Zinc also has a role in exported hydrolytic enzymes that break down external organic debris.

3.3. Summary

The key nutrient elements need to be supplied in prescribed stoichiometric ratios to ensure the optimal performance of the cell in the face of environmental changes. Active regulation, via negative feedback, of the uptake of essential elements by phytoplankton cells has been demonstrated for Cu (Sunda and Huntsman, 1995c), Zn (Sunda and Huntsman, 1992), Fe (Harrison and Morel, 1986) and Mn (Sunda and Huntsman, 1986) and for the macronutrient components nitrate (Gotham and Rhee, 1981a) and phosphate (Gotham and Rhee, 1981b).

The pathways are strongly interlinked (Figure 3) – defining a complex internal economy for the use and re-use of the fundamental ingredients. Williams and da Silva argue strongly that the mesh of interactions within the cell constitute a homeostatic system dominated by the regulatory role of the trace metals. Strong interactions have been observed between the cycles of the essential trace metals within phytoplankton cells. This internal homeostasis could provide a microcosm for a regulatory web in the wider environment if these single-celled organisms are able to impress their requirements on the oceans on a global scale. It has been proposed (Williams, 1996), for example, that the internal regulation of nitrogen metabolism within single-celled organisms is reflected in the global regulation of the nitrogen cycle. Since nitrogen metabolism is itself controlled by enzymes, most of which require a metal cofactor, this relates closely to the concept of trace metal homeostasis. It is this connection that we will explore in the following sections.

The involvement of inorganic chemistry in the evolution of the cell is neatly summarized by da Silva and Williams (1991):

> . . . one is struck first by the extent of the total involvement of inorganic elements in structural and mechanical functions; electrochemical control; acid-base and redox catalysis; generation, transmission, and storage of energy; transmission of messages and triggering effects; and so on, very much in line with the chemical possibilities for individual elements.
>
> However, there is remarkably little in common between the functions of different elements. Such a complete separation of element function is not expected from chemistry judged directly from the periodic table, since neighbouring chemical elements in the table are often similar in properties . . . For example, copper, zinc, and iron are good Lewis acids, and each is used to some degree in biology; however, at first sight puzzlingly, zinc has been selected overwhelmingly above all others for this function.

> . . . so there must be good biological (not chemical or biochemical) reasons for such an individualized choice which biochemical analysis by itself does not reveal. Some of these features will have arisen, of course, since biological evolution, but not chemistry, has been very restricted by the distribution of the elements themselves in the universe and on earth. (Reproduced with the permission of the copyright holders from: da Silva, F. J. J. R. and Williams, R. J. P. (1991). "The Biological Chemistry of the Elements". Clarendon Press, Oxford.)

Oceanic phytoplankton, constrained by this inbuilt biochemistry, have strategies selected for optimizing the acquisition and use of the elements essential for growth and reproduction. The biological requirements for these elements will have been honed through evolution in at least four interrelated ways:

- internal – by refining their storage, use, recycling and linkages with other element cycles within the cell;
- external – by modifying the speciation, uptake and sequestration of the elements at the cell surface;
- interspecific – by providing many similar but distinct solutions to internal and external metal regulation, thereby providing an array of species capable of responding optimally to changes in the availability of the elements;
- collective – by linking together many species to use the processing power of the community or the ecosystem to permit greater flexibility in the use and recycling of the elements in the face of changes in their availability.

4. THE INTERNAL ECONOMY AND THE NEAR-FIELD CHEMISTRY

The central roles played by the essential trace metals, and their low concentrations in sea water together suggest that their availability (or the lack of it) could limit primary production in the modern ocean. There is strong evidence that this is the case, obtained primarily from experimental studies with phytoplankton cultures, for Zn, Mn and Fe (Brand *et al.*, 1983) and for Cu and Cd (Brand *et al.*, 1986). Indeed most of the essential trace metals are present in such low concentrations in sea water that they can sometimes be considered as co-limiting (Morel *et al.*, 1991). This evidence will now be reviewed and its implications considered in the light of the evolutionary development of the trace metal systems within prokaryotic and eukaryotic cells.

Phytoplankton exhibit immense phylogenetic diversity (Cavalier-Smith, 1993; Falkowski and Raven, 1997; Lee, 1999) and would be expected to show a wide range of responses to the availability of the essential elements.

In terms of species numbers the dominant class of eukaryotes is the Bacillariophyceae (diatoms) which contains over half of the known marine species (Cavalier-Smith, 1993; Falkowski and Raven, 1997). This class has therefore attracted most attention in the development of experimental studies of the nutritional requirements of marine phytoplankton (Sunda, 1989).

4.1. Trace metal uptake by phytoplankton

4.1.1. *Theory*

Bearing in mind the different characteristics of prokaryotes and eukaryotes (Table 1), the uptake of trace metals by phytoplankton cells can be considered in three stages (Figure 4):

1. Transport of metal species to cell surface (diffusion);
2. Binding to a biologically-produced ligand (sequestration or capture);
3. Transfer of complex across cell membrane (internalization).

We need to know what factors control each of these stages, and to identify those stages likely to be rate limiting.

The treatment of Morel and his co-workers (Morel *et al.*, 1991) will be used here. Overall, the uptake process generally follows Michaelis-Menten kinetics, typical for enzyme-mediated reactions, as shown for Fe and Mn (Sunda and Huntsman, 1985, 1986; Hudson and Morel, 1990):

$$\rho = \frac{\rho_{\max}[M']}{K_\rho + [M']}$$

where ρ is the uptake rate, K_ρ is the half-saturation constant and $[M']$ is the available metal concentration. The maximum uptake rate ($\rho_{\max}$) can be varied (by a factor of 20–30) by the phytoplankton cell by adjusting the surface ligand concentration ([L1], Figure 4), depending on the degree of limitation of the trace metal (Sunda and Huntsman, 1985; Harrison and Morel, 1986). The processes involved are discussed in detail in terms of trace metal homeostasis by Williams and da Silva (da Silva and Williams, 1991; Williams and da Silva, 1996). For the first row transition elements of interest, $[M']$ is essentially the concentration of free metal ion and kinetically labile complexes adjacent to the cell surface. This will depend upon the strength and the rate of dissociation of dissolved complexes of the metal in solution (ML_n, Figure 4) relative to the strength and rate of

Figure 4 The uptake of essential trace metals at the cell surface. (1) The bulk solution where the metal (M) is complexed by various inorganic and organic ligands (L_n). (2) The diffusion zone where complexes interact with ligands that are either attached to the cell surface (L1) or released into the bulk solution (L2). (3) Transport zone where the metal–ligand complex is taken into the interior of the cell. The ligands are recycled and returned to the cell surface (dashed lines).

formation of ML1 complexes at the cell surface (Turner and Whitfield, 1979b, 1980; Turner, 1986). In the case of iron, colloidal particles ($<0.1\,\mu$m) could be accessible if the cell was capable of pinocytosis or phagocytosis (Barbeau and Moffett, 2000).

K_ρ is assumed to be fixed for a given trace metal and a given phytoplankton species. Its value depends upon the rates of ligand exchange (k_{-L}, k_L), and on the rate of transport into the cell (k_{in}).

$$K_\rho = \frac{k_{-L} + k_{in}}{k_L}$$

When a trace metal is limiting, the surface is undersaturated and the numerical value of $[M']$ is far smaller than that of K_ρ. At steady state:

$$\rho^{ss} = \frac{k_{in}[L1]^{\max}}{K_\rho}[M']$$

where $[L1]^{\max}$ is the maximum concentration of surface ligands that the cell can produce. Under limiting conditions, there will also be minimal release of M so that

$$K_\rho \approx \frac{k_{in}}{k_L}$$

and therefore:

$$\rho^{ss} = k_L[L1]^{\max}[M']$$

The growth rate (μ) is given by:

$$\mu = \rho^{ss}/Q$$

where Q is the internal concentration or cell quota for the trace metal (see Table 6 for data from the marine diatom *Thalassiosira weissflogii*). The uptake rate is a positive function of Q (Droop, 1973). In laboratory cultures, *Thalassiosira weissflogii* cells can also adjust their Q values in

Table 6 Concentration of trace metals in *Thalassiosira weissflogii* growing at 90% of the maximum growth rate.[a, b]

Metal	$Q_{0.9}$ (10^{-18} mol cell^{-1})	Metal:C (μmol mol^{-1})	Reference
Mn	80	6.7	Harrison and Morel, 1986
Fe	80	6.7	Harrison and Morel, 1986
Co	30	2.5	Price and Morel, 1990
Ni[c]	20	1.7	Price and Morel, 1991
Zn	50	4.2	Sunda and Huntsman, 1992
Cd	20	1.7	Price and Morel, 1990

[a]Reproduced from: Morel, F. M. M., Hudson, R. J. M. and Price N. M. (1991). Limitation of productivity by trace metals in the sea. *Limnology and Oceanography*, **36**, 1742–1755, with the permission of the copyright holders, the American Society for Limnology and Oceanography.
[b]The cells contained 12×10^{-12} mol C per cell. Cell volume 75×10^{-15} l (5.6 μm radius).
[c]Cells in the ocean can obtain 30–50% of their nitrogen from urea (McArthy *et al.*, 1977; Harrison *et al.*, 1985).

response to changing external trace metal concentrations (Morel *et al.*, 1991). Limitation occurs when Q can be reduced no further. This minimum cell quota (Q_{min}) varies from species to species. Adaptations of oceanic species to low ambient Fe, Zn and Mn concentrations (Brand *et al.*, 1983) probably arise primarily from their ability to engineer a low Q_{min}.

Combining the previous two equations gives:

$$\mu = [M']k_L[L1]^{max}/Q$$

that brings together the four parameters controlling the growth rate under trace metal limitation: available metal concentration $[M']$, the ligand exchange rate constant k_L, the maximum attainable cell surface ligand concentration $[L1]^{max}$, and the cell quota of the trace metal Q. The cell must therefore optimize the available metal concentration and the performance of the surface ligand. Since this discussion is concerned with the roles of the essential trace metals in the internal metabolism of the cells, as well as their roles at the cell surface, the transport into the cytoplasm must be considered as an integral part of the uptake process.

4.1.2. *Available metals*

The first concern here is the speciation of the trace metals in the bulk solution outside the diffusion zone adjacent to the cell. The inorganic speciation of the essential trace metals is quite well characterized (Turner *et al.*, 1981; Byrne *et al.*, 1988) and can be rationalized with respect to the complexation field diagram (Figure 1), where the essential trace metals are closely grouped. With the exception of iron, these trace metals should exhibit quite a high proportion of the free divalent metal ion in solution (10–50% of the total concentration) and the inorganically complexed species should be sufficiently mobile to release free metal within the diffusion zone (Turner and Whitfield, 1980; Turner, 1984, 1986). Iron is present as Fe (III) at equilibrium in the oceans and it is strongly hydrolysed. It is the only element in the oceans whose particulate concentration is greater than its dissolved concentration. The equilibrium concentration of Fe^{3+} resulting from these inorganic interactions is of the order of 10^{-20} M (Morel and Hudson, 1985).

However, direct measurements in sea water using electrochemical techniques indicate that a large proportion (usually >97%) of the total metal in solution is held in strong organic complexes. This has been shown for zinc (van den Berg, 1985; Bruland, 1989), cadmium (Bruland, 1992), copper (van den Berg, 1982; Coale and Bruland, 1988), nickel (van den

Berg and Nimmo, 1987), iron (Gledhill and van den Berg, 1994; Rue and Bruland, 1995; van den Berg, 1995; Wu and Luther, 1995; Boye and van den Berg, 2000) and cobalt (Zhang *et al.*, 1990). With the exception of iron siderophores and cobalamin (vitamin B_{12}), these complexes are apparently not available for phytoplankton uptake. The presence of organic complexes is a mixed blessing, since they suppress the concentration of available copper (thereby reducing its toxic effects), but they also reduce the available concentrations of zinc. As zinc also has the potential to be a toxic element, the overall impact of this complexation on the viability of phytoplankton is unclear. The impact of organic complexes on the availability of iron is also a matter of some debate (Boyle, 1997; Johnson *et al.*, 1997a, b; Luther, 1997; Sunda, 1997). The organic complexation does serve to keep the iron in solution, whereas it would otherwise be very rapidly scavenged onto particles. The organic complexes also provide a potential site for the photoreduction of Fe (III) to Fe (II) that is more readily assimilated, provided that it can be accessed in a timescale that is short compared with the oxidation rate (i.e. on the scale of minutes to hours). In practice ML_n (Figure 4) represents the inorganic complexes in solution.

Extensive experimental work on phytoplankton cultures, using metal buffers (e.g. EDTA), has shown that the free metal ion concentrations calculated from speciation models can provide an excellent guide to the availability of the trace metals in solution (Morel and Hudson, 1985; Morel *et al.*, 1991; Sunda, 1989; Sunda and Huntsman, 1998b). This has given rise to an equilibrium-based "free ion model" for the uptake of trace metals (Campbell, 1995) which assumes that the uptake of trace metals by the cell (zone (3), Figure 4) is sufficiently slow that it is rate limiting and the reactions in zones (1) and (2) can reach equilibrium. The free metal ion at the cell surface therefore represents the metal available for uptake, and the concentration of ML1 at the surface is at equilibrium with this concentration.

4.1.3. *Metal capture and sequestration*

In the situation where the assimilation of the trace metal is rapid (i.e. the cell is using the trace metal as quickly as, or possibly more quickly than, it is provided), the potential rate-limiting steps become the diffusion of M and the ML_n complexes to the cell surface and the rate of the complexation reaction producing the carrier complex ML1 (Eide, 1997). This situation has been investigated by Hudson and Morel (Morel *et al.*, 1991; Hudson and Morel, 1993; Hudson, 1998). There is strong evidence that carrier complexes are involved in trace metal uptake (Sunda and Huntsman, 1998b).

The role of carrier complexes suggests that:

1. the uptake process will be saturable since only a finite number of L1 molecules can be accommodated at the cell surface[2] and
2. that competitive inhibition of essential metal uptake by non-essential metals can occur.

The surface binding of iron and the transport of the complex across the membrane was studied by Hudson and Morel (1990). Ligand optimization requires synthesis of the maximum concentration of readily exchangeable ligands with high binding constants at the cell surface. Exchangeability is primarily a characteristic of the cation since k_L is directly related to k_{H_2O} (Morel *et al.*, 1991), the intrinsic rate of water exchange at the metal ion surface (Table 5). It is intriguing to note that the log k_L values of the essential trace metals correlate with the available metal concentrations in surface waters (expressed as log[M'], Figure 5, and Table 5) (Morel *et al.*, 1991; Hudson and Morel, 1993). This suggests that the kinetic lability of

Figure 5 The relationship between the rate of ligand exchange (k_L, Table 5) and the concentration of metal ion not complexed by organic matter in oceanic surface waters. Replotted from data in Hudson and Morel (1993) as summarized in Table 5.

[2]For example in the North Pacific, with an available iron concentration of 5×10^{-11} M, a 10 μm diameter cell would require a minimum density of 4×10^{-12} M cm^{-2} to maintain growth. The molecules must be relatively small (<1 kDa, compare siderophores) for that number to fit on the membrane (Morel *et al.*, 1991).

the carrier complexes might control the availability of these trace metals and their removal from the surface ocean:

> The inverse relationship between $[M']$ and k_L may reflect the interdependence of the properties of metal transport systems, the metabolic requirements of phytoplankton and the oceanic concentrations of the elements. (Hudson and Morel, 1993)

The carrier molecules do not appear to be specific. For example the $-\log K_\rho$ values measured in the marine diatom *Thalassiosira pseudonana* are 7.1 (Mn), 7.5 (Zn) and 8.1 (Cd) (Sunda and Huntsman, 1996). This limits the degree of kinetic control on the specific uptake of essential metals and may explain in part the inadvertent but efficient uptake of Cd. The characteristics of the carrier are most important in defining the rates of transport across the membrane and release into the cytoplasm (Hudson and Morel, 1993). The uptake of micronutrients requires many transport molecules working far from saturation. These molecules must be small because of space limitations, but their metal exchange kinetics do not need to be fast.

Diffusion limitation (Wolf-Gladrow and Reibesell, 1997; Hudson, 1998) can occur if the readily accessible free ions are consumed at the cell surface more rapidly than they arrive. This diffusion limitation is confined to the layer immediately adjacent to the cell surface and external turbulence and cell movement affect the thickness of the zone only slightly. It would be expected that under conditions where micronutrients may be limiting, the organisms would increase the effectiveness of their transport systems to the point where diffusion across this layer limits the rate of uptake. Hudson (1998) has shown that in most culture experiments at natural trace metal concentrations the observed flux is close to the diffusion-limited flux (Table 7), and diffusion limitation could occur for both Zn and Fe. For Mn neither transport nor growth rates approach diffusion-limited values. Rather the interaction between Mn and Cu controls growth through its effect on Mn transport and intracellular utilization (Sunda and Huntsman, 1983). Some artefacts are observed in experiments with Cu^{2+}, probably because of redox reactions converting Cu (II)EDTA complexes to Cu (I)EDTA complexes at the cell surface.

Prokaryotes have an alternative strategy and release complexing agents (siderophores, represented by L2 in Figure 4) into solution (Martinez *et al.*, 2000). The performance is nicely summarized by da Silva and Williams (1991, p. 323):

> The design of these reagents is such that they are soluble in water when not bound by iron, but on binding they turn inside out and become able to pass through lipid membranes.

Table 7 Diffusion from the bulk medium to the cell surface as a controlling mechanism.[a]

Metal	Species	Ratio of observed flux to diffusion-limited flux	Reference
Fe^{3+}	*Thalassiosira weissflogii*	0.2	Hudson and Morel, 1990
	Thalassiosira oceanica	0.038	Sunda and Huntsman, 1992
Zn^{2+}	*Thalassiosira pseudonana*	0.28	Sunda and Huntsman, 1992
	Thalassiosira oceanica	0.61	
	Emiliania huxleyi (BT6)	0.35	
	Emiliania huxleyi (A1323)	0.37	
Cu^{2+}	*Thalassiosira pseudonana*	31[b]	Sunda and Huntsman, 1995c
	Thalassiosira oceanica	17[b]	
	Emiliania huxleyi	13[b]	
	Thalassiosira weissflogii	0.034	
Mn^{2+}	*Thalassiosira pseudonana*	0.003	Sunda and Huntsman, 1996
Cd^{2+}	*Thalassiosira pseudonana*	0.001[c]	Sunda and Huntsman, 1996
Zn^{2+}	*Thalassiosira pseudonana*	0.007[c]	

[a]Reproduced from: Hudson, R. J. M. (1998). Which aqueous species control the rates of trace metal uptake by aquatic biota? Observations and predictions of non-equilibrium effects. *Science of the Total Environment* **219**, 95–115, with the permission of the copyright holders, Elsevier Science.
[b]Indicates probability of uptake via surface reduction of Cu (II)-EDTA complexes to give Cu (I) transport.
[c]Via Mn transporter.
The *Thalassiosira* cells are approximately 30 μm in diameter and the *Emiliania huxleyi* cells approximately 15 μm in diameter. Oceanic data for Fe, Mn, Zn and Ni are summarized by Hudson and Morel (1993). They suggest that Fe and Zn concentrations are close to or less than the diffusion limit.

The widespread broadcasting of ligands is rather a wasteful process unless the number of cells is sufficiently high for the individuals to gain a mutual advantage from the communal release (Völker and Wolf-Gladrow, 1999), or unless it reduces the availability of Fe for competing species. Cyanobacteria are generally much smaller than eukaryotic phytoplankton and their larger surface area:volume ratio (Table 1) favours the uptake of dissolved components (Hutchins, 1995). They also have the capability of generating significant quantities of specific siderophores, especially for iron, and thereby altering the chemistry of the ocean on a large scale (Moffett *et al.*, 1997). The complexes have the dual benefit of increasing the availability of iron (Rue and Bruland, 1995, 1997) while decreasing the availability of copper (Moffett *et al.*, 1990, 1997) which, as we have seen, is especially toxic to prokaryotes. Specific receptors trap the incoming

siderophore complexes on the cell surface and pass them unchanged through the cell membrane.

Until recently it was generally thought that eukaryotic phytoplankton utilized only surface attached ligands to trap free metal ions from solution (L1, Figure 4). These ligands tend to be non-specific cell surface enzymes such as ferri-reductase (Hutchins *et al.*, 1999a). However, there have been observations of the release of specific complexing agents directly into solution by dinoflagellates (Trick *et al.*, 1983), coccolithophorids (Boye and van den Berg, 2000) and diatoms (Hutchins *et al.*, 1999b). These compounds are not siderophores but are intracellular iron-binding molecules (e.g. porphyrins) that are normally produced within the cells for other purposes (Geider, 1999).

4.1.4. *Minimization of the cell quota (Q)*

The minimization of Q depends upon the ability of the cell either to substitute other trace metals for essential functions if the metal of choice is not available, or on the cell's propensity for the efficient internal storage and recycling of elements. The most extreme example is provided by iron because of its very low availability outside the cell and the wide range of requirements for its use inside the cell. Iron is one of the core elements built into the central cell metabolism during the early stages of evolution. It remains of central importance to prokaryotic and eukaryotic cells (see Tables 2 and 4), although the iron requirements of prokaryotes are higher than those of eukaryotes because of the significant degree of substitution for iron functions by zinc and copper in the later stages of evolution.

The demands for iron are high. At least 15% of the cell Fe is required to support nitrate and nitrite reductase, while nitrogen fixation in cyanobacteria makes even larger demands. For amino acid formation the cells would require 40% more iron if NO_3^- rather than NH_4^+ was used as the nitrogen source. The anticipated high Fe dependence of oceanic diatoms is not found in culture experiments (Morel *et al.*, 1991), suggesting a considerable degree of parsimony in iron utilization. It would appear that oceanic diatoms have pushed their minimum cell quotas (Q_{min}), particularly for Fe, to very low levels (Sunda *et al.*, 1991; Sunda and Huntsman, 1995b) since they can function with only a third of the cell quota of iron required by their coastal cousins. *Thalassiosira weissflogii* can use an alternative strategy in combating Zn deficiency by using Co and Cd as functional substitutes within the cell (Price and Morel, 1990; Cullen *et al.*, 1999).

For Fe and Zn, the ambient concentrations in open ocean surface waters are low enough for diffusion to limit their ability to sustain maximal

growth. Where there is diffusion limitation, the trace metal requirements and viability of cells will depend upon their size. The limiting concentration for a typical cyanobacterium would, for example, be one twentieth of that experienced by a typical diatom (Hudson and Morel, 1993). This relationship could have significant implications for ecosystem structure since the export of particulate matter from the surface layers is sensitive to the size of phytoplankton, and of related grazers.

4.2. Interactive influences

4.2.1. *Background*

It was noted earlier that the essential trace metals (Mn, Fe, Co, Ni, Zn, Cu) are not as unequivocal as the major cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) and the structural nutrient elements (C, N, P, S) in the functions that they perform within the cells. The essential trace metals form a coherent group in the complexation field diagram (Figure 1) and this provides a considerable degree of flexibility in their biological use. Apart from certain core functions within the cytoplasm, there has been a significant degree of substitution and replacement of some functions of Fe and Mn, especially by elements (principally Zn and Cu) that were released into solution as the oceans became progressively oxygenated. Within the cells, the cycles of the elements interact (Figure 3) so that it is often difficult to attribute the success or failure of a cell to any single cause. Indeed many enzymes require the cooperative presence of several different metals. This intertwining and interlinking of the metal cycles gives rise to the possibility of homeostatic control of the cell's acquisition and utilization of trace metals (da Silva and Williams, 1991; Williams and da Silva, 1996).

Also, it appears that the uptake mechanisms in phytoplankton are working close to their optimum levels in the face of very low concentrations of the available metal species. This has given rise to suggestions that the productivity of the oceans may be co-limited by a suite of trace metals as well as running close to the depletion of the major nutrients (N and P) in the upper layers of the ocean (Morel and Hudson, 1985; Zachos *et al.*, 1989; Bruland *et al.*, 1991; Morel *et al.*, 1991). The limitation of phytoplankton growth and productivity by a simultaneous shortage of Zn, Mn and Fe would be more severe than that imposed by any single metal (Brand *et al.*, 1983), since the cell will have fewer options for substituting for the functionality of the metals that are in short supply.

Together these observations suggest that the impact of a given metal *M* upon the physiology of a cell will be influenced by the presence and the concentrations of other metals in the surrounding solution. These

interactions may augment the impact of *M* on the performance of the cell (synergism) or they may oppose or inhibit the impact of *M* on the performance of the cell (antagonism). The impact of pollution on phytoplankton growth, particularly in coastal waters, has stimulated a large amount of research on the response of phytoplankton to different mixtures of trace metals. This work has enabled some important observations to be made on the potential impact of pollutants on phytoplankton productivity. It has also provided valuable insights into the physiology of phytoplankton cells and their responses to the availability of trace metals, and the interactions between trace metals (Table 8).

The patterns of interaction (Figure 6) have been discussed in detail (Sunda and Huntsman, 1983, 1995a, 1996; Sunda, 1989; Bruland *et al.*, 1991). Three examples will be used to illustrate the processes at work and to give some indication of the complexity of the natural system.

4.2.2. *Cobalt and zinc*

The substitution of Co for Zn may be considered as a benign interaction between the metals, since it provides phytoplankton that can use it with an additional means of combating trace metal limitation (Sunda and Huntsman, 1995a).

Table 8 Studies of interactions between essential metals in phytoplankton cultures.

Elements	Species	Reference
Cu-Mn	Estuarine and oceanic species of diatoms *Thalassiosira*	Murphy *et al.*, 1984; Sunda, 1976; Sunda *et al.*, 1981; Sunda and Huntsman, 1983
Cu-Fe	Diatoms	Murphy *et al.*, 1984
Cu-Zn	Silica uptake mechanism of diatoms	Reuter and Morel, 1981
Cu-Zn	Ciliates *Balanion* sp.	Stoecker *et al.*, 1986
Cd-Fe	Diatom *Thalassiosira weissflogii*	Foster and Morel, 1982; Harrison and Morel, 1983
Mn-Fe	Diatom *Thalassiosira weissflogii*	Harrison and Morel, 1986; Murphy *et al.*, 1984
Mn-Zn	Mixed populations in a field study	Sunda, 1987
Co-Zn	*Thalassiosira pseudonana*	Sunda and Huntsman, 1995a
	Thalassiosira oceanica	
	Emiliania huxleyi	Cd-Zn-Mn
Coastal diatoms		Sunda and Huntsman, 1996
		Sunda and Huntsman, 1998a

Figure 6 Patterns of interaction between the essential trace metals. Dark arrows indicate the direction of a deleterious impact and light arrows the direction of a beneficial impact. (Adapted from Bruland *et al.*, 1991.)

Prokaryotes have little requirement for Zn since their fundamental internal mechanisms evolved in an anoxic ocean where zinc was less readily available. In the primeval anoxic ocean Co (II) would be more soluble than Zn. For example, anoxic interstitial waters have at least 10-fold lower Zn (Jacobs *et al.*, 1985) and 10-fold higher Co (Dyrssen and Kremling, 1990) than overlying waters. There is an unusually high requirement for Co in methanogenic archaebacteria (da Silva and Williams, 1991). In eukaryotes, by contrast, zinc is widely used and is present in several hundred enzymes (Vallee and Galdes, 1984; Vallee and Auld, 1990). It is possible for Co to substitute for Zn in carbonic anhydrase (Morel *et al.*, 1994) and other enzymes (Vallee and Galdes, 1984).

Cobalt (and cadmium) can substitute for zinc in some marine diatoms and maintain growth rates at low available zinc levels (Price and Morel, 1990; Sunda and Huntsman, 1995a). For example, in *Thalassiosira pseudonana* and *Thalassiosira oceanica*, Co could largely meet the physiological demands normally provided for by the cell's Zn intake (Sunda and Huntsman, 1995a). However, not all diatom species behave in the same

way and recent studies have shown that there is no apparent substitution of Co for Zn in *Chaetoceros calcitrans* (Timmermans, personal communication). Only partial substitution has been noted for the coccolithophorid *Emiliania huxleyi* (Price and Morel, 1990).

Culture experiments on *Thalassiosira* species (Sunda and Huntsman, 1995a) show rapidly increased Co uptake rates as ambient Zn levels decline. Cellular Zn levels are actively regulated. Nearly constant internal zinc levels can be maintained over a wide range of external Zn^{2+} concentrations by negative feedback regulation of the supply of surface complexation sites (L1, Figure 4) and hence the transport capacity (da Silva and Williams, 1991; Sunda and Huntsman, 1992). However, a limit to this regulation is reached when the external free Zn^{2+} concentration is so low ($<10^{-13}$ M) relative to the cells' requirements that the supply is limited by diffusion across the boundary layer (Table 7). The uptake of Co^{2+} increases rapidly as this limit is approached until its supply to the cell surface is in turn limited by diffusion rates. The Co uptake rates by diatoms in these experiments are more than a hundred times greater than is required to meet the cells' vitamin B_{12} requirements (da Silva and Williams, 1991). There is evidence that Cd as well as Co can substitute for Zn in diatoms (Price and Morel, 1990; Cullen *et al.*, 1999) and this has been put forward as an explanation of the low Zn cell burdens necessary for growth in coastal (neritic) species (Sunda and Huntsman, 1992).

The varying requirements for Zn and Co in different species give rise to some interesting speculation about the role of the availability of trace metals in structuring marine communities (Table 9). Cobalt replacement for Zn could be seen as an adaptive strategy for growth in the open ocean where Zn levels are low (Sunda and Huntsman, 1995a). Both Zn_T concentrations and Zn:Co ratios vary widely and this could affect the relative viability of diatoms and coccolithophorids, for example. Unfortunately,

Table 9 The requirements of phytoplankton species for cobalt and zinc.[a]

Species	Co	Zn
Synechococcus bacillaris (cyanobacteria)	Low requirement	Not required
Emiliania huxleyi (coccolithophore)	Requirement can be partially met by Zn	Required
Thalassiosira pseudonana *Thalassiosira oceanica* (diatoms)	Required	Requirement could be largely met by Co

[a]Compiled from data in Sunda and Huntsman (1995a).

Co levels are usually even lower than those of Zn with a maximum Co:Zn ratio of about 0.4. It also appears that Co (II) can be oxidized to Co (III) by microbially mediated processes and oxidatively scavenged from sea water in a manner similar to Mn removal (Lee and Fisher, 1993a), weakening the case for a long-term adaptive strategy.

4.2.3. *Cadmium, manganese and zinc*

In open ocean waters the Cd concentration is usually higher than that of Co, but lower than that of Zn. The possibility therefore arises of Cd substitution for Zn and also, in polluted conditions, for Mn. Experiments with the coastal diatom *Thalassiosira pseudonana* using EDTA buffer media have shown that the cellular cadmium quota (Q_{Cd}) is directly related to Cd^{2+} concentrations and inversely related to Zn^{2+} and Mn^{2+} ion concentrations in the external medium (Sunda and Huntsman, 1998a). At least two separate micronutrient transport systems appear to be at work, one for Zn uptake and one for Mn uptake.

At high Zn^{2+} ion concentrations, Q_{Cd} is almost exclusively controlled by the Mn system that is under negative feedback. This increases μ_{max} for Cd 10-fold as the Mn^{2+} ion concentration falls from 7.9×10^{-8} M to 6.3×10^{-9} M, but can increase it no further. As Zn^{2+} ion concentration is decreased from 10^{-10} M to 10^{-12} M, there is a large increase in Cd uptake rates. This is similar to the Zn-induced uptake of Co described above. The largest increase occurs when the Zn^{2+} ion concentration falls below 10^{-11} M. This is the region where Zn uptake becomes limited by diffusion. Cd uptake rates may be significantly higher in the open ocean than in coastal waters, despite the lower Cd levels, because the low ambient levels of Mn and Zn will induce these additional pumps.

To test the idea, Sunda and Huntsman (1998a) used data on the relative concentrations of Zn, Cd and Mn in Pacific surface water, together with the data from the culture experiments to calculate the Q_{Cd} values and the Cd:C ratios that would be expected in this environment. Using the known C:P ratios in these organisms they were then able to calculate the Cd:P ratios. The mean value obtained was $191 \pm 74\ \mu mol\ mol^{-1}$. This compares surprisingly well with the ratio of $240\ \mu mol\ mol^{-1}$ calculated from correlations between dissolved Cd and dissolved P concentrations in deep water (Section 6.5.1).

4.2.4. *Manganese, copper and zinc*

So far the substitutions concerned have been benign, with the intruding trace metal acting as a surrogate for the trace metal in short supply and

thereby increasing the range of options available for the phytoplankton cell. There are more aggressive situations where the intruding metal damages the cell or decreases the potential for growth and survival. The toxicity of Cu can be relieved in the presence of Mn in cultures of *Thalassiosira pseudonana* (Sunda and Huntsman, 1983). Conversely, the growth of the diatom can be inhibited by the interference of Cu with the Mn metabolism of the cell. Growth rates are similar at a given free Mn^{2+}:Cu^{2+} ratio over a wide range of concentrations. This suggests that there is a threshold level of the toxic metal relative to the nutrient metal, above which it can compete effectively at the metabolic site. Near maximum growth rates are observed when the ratio of free Mn^{2+}:Cu^{2+} is $>10^{3.5}$. Complete inhibition of growth is observed when the ratio is $<10^{-1.2}$.

Bruland *et al.* (1991) have considered the implications of these findings for diatoms growing in the surface sea water in the North Pacific gyre. In the surface layer, they calculated the free Mn^{2+}:Cu^{2+} ratio to be 10^5, so that free Mn levels are high and organic complexation of the copper has effectively suppressed its toxicity and optimum growth is possible. However, for waters sampled from below the euphotic zone between 200 and 400 m, the ratio of free Mn^{2+}:Cu^{2+} is approximately $10^{0.7}$ and is therefore in the range of potential inhibition of growth by Cu. This effect could explain in part the initial inhibitory effect of water upwelling from these deeper layers on growth (Sunda *et al.*, 1981; Sunda, 1989). Once in the surface layer, the water could become conditioned biologically (Barber and Ryther, 1969) either by the production of Cu-chelating ligands to reduce the free Cu^{2+} level, or by the photoreduction of Mn (IV) via organic complexes to increase the free Mn^{2+} level.

A similar calculation can be carried out for the inhibition of the cellular functions of Zn by competition from Cu. Here again, using the North Pacific central gyre, Bruland *et al.* (1991) calculated the free Cu^{2+}:Zn^{2+} ratios in the surface water and in the deeper water. The results were compared with the observed effects of Cu and Zn on the growth of a grazing ciliate (*Balanion* sp.) using the data of Stoecker *et al.* (1986). If the free metal ion concentrations were the prime concern, the surface waters would have allowed reasonable (but not optimal) growth of the ciliate, but the deeper waters would have inhibited growth. Again it is the free metal ion ratios rather than the absolute concentrations that are influencing the viability of the animals. This raises the interesting possibility that some of the effects on primary production ascribed to trace metals added to natural samples could arise from their impact upon the grazing animals (Coale, 1991).

The impact of trace metals at high (pollutant) concentrations falls outside the main sphere of interest of the present review. However, these studies can yield some interesting surprises. This is neatly illustrated

by Morel *et al.* (1991) who use information on the interactions in the Zn-Mn-Cu system:

> An amusing and extreme example of competitive uptake inhibition is provided by the "zinc squeeze" phenomenon (Sunda, 1989). At low [Zn], Zn may be a limiting nutrient and at high [Zn] it is a toxicant due, for example to interference in the metabolism of other essential metals. Because Cu competitively inhibits Zn uptake, Zn requirements are elevated at high [Cu]. Conversely, Zn competitively inhibits Mn uptake so that at low ambient [Mn], even relatively low [Zn] can interfere with the Mn nutrition of cells. At high [Cu] and low [Mn] the result can be a paradoxical situation in which Zn is simultaneously limiting and toxic. (Reproduced from: Morel, F. M. M., Hudson, R. J. M. and Price, N. M. (1991). Limitation of productivity by trace metals in the sea. *Limnology and Oceanography*, **36**, 1742–1755, with the permission of the copyright holders, The American Society for Limnology and Oceanography.)

5. REDFIELD RATIOS – THE GLOBAL IMPRINT OF PHYTOPLANKTON

The previous section considered the interaction between phytoplankton and essential trace metals in their immediate chemical environment as revealed by culture experiments. The focus was on the internal functioning of the cell and on the interactions between cellular processes and the near-field chemistry of the elements in sea water. The implications of these interactions on the far-field chemistry of the ocean will now be considered. This link is described by Williams (1996) as "the internal physiological control setting the environmental parameters".

5.1. Macronutrient elements

During photosynthesis in the open ocean, the macronutrient elements, nitrogen and phosphorus, are taken up in stoichiometric molecular ratios dictated by the physiology of the phytoplankton. In mesotrophic areas, nitrogen and phosphorus are often drawn down together to extremely low levels at which experimental studies suggest that the growth of the cells might be constrained (Hecky and Kilham, 1988). In these circumstances, inorganic nitrogen reservoirs in the sea water are usually exhausted before phosphate reservoirs (with a notable exception being observed in the eastern Mediterranean Sea). Nitrogen is therefore considered as the "proximate (or local) limiting nutrient" (PLN) (Tyrell, 1999).

In culture there is significant variability in the C:N:P ratios in phytoplankton, both between species and within species (Moal *et al.*, 1987), depending on the balance struck between synthesis and storage. N:P ratios can range from 5 to 19 (with more extreme values under conditions where one of the nutrients is limiting, or is present in high concentrations), and C:N ratios from 4 to 17. However, from studies of the average composition of phytoplankton samples taken at sea and of the composition of sea water from the euphotic zone (Harvey, 1926; Redfield, 1934, 1958; Redfield *et al.*, 1963) a mean C:N:P ratio of 106:16:1 was proposed, which is considered as the classic Redfield ratio.

Lower N:P ratios of 14.7 (and higher C:P ratios up to 135) are observed in deep ocean waters (Codispoti, 1989) because of the presence of denitrifying bacteria. Tyrrell (Tyrell and Law, 1997; Tyrell, 1999) has investigated the mechanisms underlying the regulation of this Redfield N:P remineralization ratio by considering the global patterns of nitrogen fixation by prokaryotic phytoplankton and denitrification by bacteria (Gruber and Sarmiento, 1997). The denitrification in the deeper water is compensated for by nitrogen fixation primarily in the euphotic zone. The benthic flux contributes <10% of natural global marine nitrogen fixation. Provided that P is not limiting, the nitrogen fixers will have an advantage when nitrate and ammonium are scarce. However, they are at a disadvantage compared with other phytoplankton when nitrate is more abundant, since N_2 fixation requires more energy and makes greater demands on the small pools of available iron (Tyrell, 1999) and diatoms have a much higher μ_{max} for the uptake of nitrogen. To convert molecular nitrogen to ammonium ions, they require large amounts of iron that is in short supply especially in areas far away from the coast. Nitrogenase has the lowest iron use efficiency of any enzyme (Raven, 1988). Twelve Fe atoms are required for photosystem I (PSI), which is also essential for nitrogen fixation. Cyanobacteria have very high ratios of PSI to PSII (25 for *Trichodesmium*) (Falkowski and Woodhead, 1992) cf. values of 0.25–1 for diatoms (Raven *et al.*, 1999), thus accentuating their iron requirements.

The suggestion is that the denitrification/nitrogen fixation balancing act adjusts the N:P ratio in sea water to that required by the physiology of phytoplankton so that optimum use is made of the available nutrients (Tyrell, 1999). Similar conclusions were reached independently by Lenton (1998b) who was also able to relate these findings to the biological regulation of atmospheric oxygen levels, since there is a Redfield ratio (Redfield, 1958; Redfield *et al.*, 1963) linking oxygen release to phosphorus uptake ($-O_2$:P = 138).

It should be stressed that the nitrogen fixers and the denitrifiers are not directly coupled in the short term, since they inhabit different areas of the ocean. The nitrogen fixers depend upon unproductive oligotrophic

conditions in the surface ocean to gain their competitive edge. The denitrifiers on the other hand depend on a rich flux of organic matter from the surface to generate the low oxygen conditions at depth in which they thrive. The system is not at steady state and significant variations have been observed in the deep ocean Redfield ratios over a decadal timescale (Paklow and Riebesell, 2000). Nonetheless, this is a striking example of the internal husbandry of the cell implanting its signature upon the composition of sea water throughout the world oceans.

Silicon is often the proximate limiting nutrient for diatoms in the open ocean (Dugdale *et al.*, 1995) when the silicon concentration is $< 5 \times 10^{-6}$ M and phosphate and nitrate are available in excess. The usual Redfield ratio for the uptake of silicon by diatoms is Si:N = 1 (Brzezinkski, 1995; Wilkerson and Dugdale, 1996).

The remarkably consistent P:N ratio throughout the deep water of the world ocean is a tribute to the biological regulation of nitrogen (Tyrell and Law, 1997; Lenton, 1998b). There is no strategy available to the biological system for regulating the phosphorus content of the oceans as there is with nitrogen. The best that can be achieved is a very parsimonious use of the supplies that are available. Phosphorus is very effectively recycled through the biological system (Volk, 1997) and only 1% of the phosphorus taken up by phytoplankton is trapped in the sediment during each ocean mixing cycle of 1000 years (Broecker and Peng, 1982). Recent studies in fresh water have shown that the recycling of phosphorus at low ambient concentrations (down to 5×10^{-11} M) is as vigorous and efficient as that of ammonium (Hudson *et al.*, 2000; Karl, 2000).

Tyrell (1999) suggests that P is the "ultimate limiting nutrient" (ULN) in the oceans (Toggweiler, 1999). He defines the ULN as:

> . . . the nutrient whose supply rate forces total system productivity over long timescales. If the river input of the ULN changes then total system primary productivity will also eventually change, perhaps over thousands of years for the case of the global ocean.

This reflects the original views of Redfield and is consistent with the concept of limitation implicit in the single stirred tank model of the ocean used to define residence times for the elements (Whitfield, 1979, 1981).

5.2. Essential trace metals

Essential trace metals also exhibit, to a lesser degree, Redfield ratios relative to the dissolved concentrations of P and Si (or sometimes for both, Appendix III). There is evidence for the bacterial acceleration of the dissolution of silicon when diatoms die or are grazed (Bidle and Azam,

1999). This may help to explain the close coupling observed between Si and other elements released from phytoplankton during respiration. However, some care must be exercised. Si and P are themselves often correlated in the deep ocean (Measures *et al.*, 1983), and advective processes can obscure the signatures of individual sources of dissolved components (Edmond, 1974). The confines of stoichiometry that provide such excellent correlations for C, N, P and to a lesser extent Si will not be so rigid for the essential trace elements since these are used primarily in enzyme systems. Therefore the cells can either store surplus metal in times of plenty (or in the extreme, sequester excess metal to prevent toxicity) or they can reduce their cell quota (Q) for the metal when trace metals are hard to come by, to a much greater degree than they can manipulate their C:N:P ratios (Moal *et al.*, 1987). The trace metals appear to act as proximate limiting nutrients and their availability can be modified significantly by biological action (Toggweiler, 1999). These factors will be considered in individual case histories for the essential trace metals.

6. CASE HISTORIES

6.1. Iron

6.1.1. *Distribution of iron in the oceans*

Stepping outside of the sequence of the elements in the first transition series the case history of iron will be considered first. This element is in great demand but in short supply (see Table 3 and Appendix I) and so the problem of iron limitation of primary production has received considerable attention and several excellent reviews have been published in recent years (Sunda, 1989; Hutchins, 1995; Wells *et al.*, 1995; Price and Morel, 1996; Turner and Hunter, 2001).

A major external source of iron for the surface layers of the open ocean is transport in aeolian dust and aerosols (Duce and Tindale, 1991) with probably >95% of the flux being delivered to the ocean surface in rain. The iron in the acid rain falling on the ocean is likely to be largely in the Fe (II) form (Zhuang *et al.*, 1992). Sunda (2001) has suggested that one consequence of the release of acid compounds into the atmosphere from dimethyl sulphide (DMS) produced by phytoplankton blooms could be an increase in the supply of this readily accessible iron source, rather than the relatively refractory mineral dust. Iron fertilization experiments have been shown to enhance the release of DMS to the atmosphere (Turner *et al.*, 1996).

The vertical profiles of iron concentration in the ocean do not fit neatly into the categories described earlier. Its chemistry dictates that it should behave as a scavenged element but in fact its vertical profiles resemble those of a recycled element (Johnson *et al.*, 1997a) (Figure 7). Johnson *et al.* have carried out a detailed analysis of the available vertical profiles of iron throughout the world ocean using samples from the North and South Pacific, the Southern Ocean and the North Atlantic. The behaviour of iron contrasts markedly with that of lead, a strongly scavenged element with a predominantly aeolian input (Flegal and Patterson, 1983). The profiles do not show any fractionation between the Atlantic and the Pacific in the deep water. They all exhibit the typical nutrient element shape (Martin *et al.*, 1989) with average concentrations at the surface of 7×10^{-11} M and below 500 m of 7.6×10^{-10} M. They show a reasonable correlation with nitrate profiles (Martin *et al.*, 1989; Johnson *et al.*, 1997a). These are unusual characteristics for an element with a reported mean oceanic residence time of <200 years (Landing and Bruland, 1987; Bruland *et al.*, 1994). There are large horizontal gradients in iron concentration, especially in the Pacific, but the surface values are not well correlated with the observed aeolian input fluxes. No correlation was observed between the particulate and dissolved concentrations of iron. The original view of an iron supply dominated by aeolian input is now considered to apply only to the subarctic Pacific (de Baar *et al.*, 1995).

Johnson *et al.* (1997a) suggest that the vertical profiles are maintained by complexation with strong iron-binding organic ligands (Rue and Bruland 1995; Wu and Luther, 1995) in deep water, which reduce the scavenging rate of iron at concentrations below 0.6×10^{-9} M. Such ligands have been found in the Atlantic and Pacific with concentrations near 0.6×10^{-9} M. Model calculations on this basis suggest that a cell quota (Q) of 5 μmol Fe mol^{-1} C (compare Table 10) and a rate constant for removal of 0.005 yr^{-1} would be consistent with the observed profiles and properties of the complexing ligands. The supply of total iron from this deep water source would provide the major flux of iron to the surface layers (cf. phosphate), with dust transport playing a secondary role (de Baar and Boyd, 1998). This would represent a major biological feedback, maintaining much higher, and more stable, total iron concentrations than would otherwise be encountered (Sunda, 1997).

The model of Johnson *et al.* (1997a) has generated considerable discussion (Boyle, 1997; Johnson *et al.*, 1997b; Luther, 1997; Sunda, 1997). There is strong evidence for the presence of organic ligands capable of complexing >95% of the inorganic Fe (III) (van den Berg, 1995; Nolting *et al.*, 1998; Witter and Luther, 1998). Two ligand classes have been identified, a strong ligand ($\log K = 13$, concentration $0.4\text{–}1.0 \times 10^{-9}$ M) in surface waters and a weaker ligand ($\log K = 11.5$, concentration

Figure 7 The nutrient-like concentration profiles of iron. ■, North Pacific; □, △, Equatorial Pacific; ○, North Atlantic. Note the uniform concentration in the deep sea. Data taken from the compilation of Johnson *et al.* (1997a, b.)

1.5×10^{-9} M) down to depths of 2000 m (Rue and Bruland, 1995). Similar behaviour for iron-complexing organic ligands has been observed over a wide geographic range (Table 11).

A combination of aeolian supply with the more conventional scavenging model (Bruland *et al.*, 1994), modified by the production of colloidal iron species, could also explain some of the features of the iron profiles (Boyle, 1997). Thorium, another strongly hydrolysed element, exhibits the same kind of pattern of deep water ^{230}Th profiles with a similar distribution in

Table 10 Measured iron quotas for plankton from different habitats.[a]

Taxon	Habitat	Fe:C[b]	Growth rate (d^{-1})	IUE[c]	n	Reference
Phytoplankton	Coastal	11	0.62	0.59	11	Price and Morel, 1996
	Oceanic	3.1	1.2	5	13	Price and Morel, 1996
	Oceanic	3.6				Sunda and Huntsman, 1995b
	Oceanic	3				Tortell *et al.*, 1996
	Oceanic	4.4				Tortell *et al.*, 1996
Cyanobacteria	Coastal	21.2	0.9	0.46	1	Price and Morel, 1996
(*Synechococcus*)	Coastal	21.2	0.9	0.46	1	Price and Morel, 1996
	Coastal	150	0.94	0.06	1	Price and Morel, 1996
	Oceanic	19				Tortell *et al.*, 1996
Heterotrophic bacteria	Oceanic	7.5				Tortell *et al.*, 1996
		9.1				
Protozoa	Coastal	10.4	1.2	6.9	2	Price and Morel, 1996
	Oceanic					

[a]Reproduced with the permission of the copyright holders from: Price, N. M. and Morel, F. M. M. (1996). Biological cycling of iron in the ocean. *Metal Ions in Biological Systems* **35**, 1–36.
[b]Fe:C in μmol Fe to mol C.
[c]IUE = iron use efficiency 10^5 mol C mol Fe^{-1} d^{-1}.

Table 11 Complexation of iron in sea water[a,b]

Location	$\log \alpha_{organic} = \log K'_{org}[L]$	$\log \alpha_{organic} = \log K'_{inorg}[L]$	Reference
Southern Ocean, Pacific sector	13.22	11.9	Nolting *et al.*, 1998
Mediterranean	11.5–14	11.6	van den Berg, 1995
North Pacific	13.7	10	Rue and Bruland, 1995
Equatorial Pacific	14.2	11	Rue and Bruland, 1997

[a]Data compiled from Nolting *et al.* (1998).
[b]K' is defined relative to the complexation of Fe^{3+}.

most ocean basins (Bacon and Anderson, 1982). No organic complexation is involved, but the ^{230}Th concentrations are controlled by a balance between uniform water column production (via the decay of ^{238}U that is an accumulated element) and a steady flux of particles exchanging ^{230}Th. The profiles observed in the Atlantic and Pacific are similar because ^{230}Th residence time is short (30 ± 10 yr) so that supplies are localized and there is no inter-ocean transport. There are also areas of the South Pacific Ocean

where the model developed by Johnson *et al.* (1997a) does not apply, because the deep water iron concentrations are lower than those indicated and iron transport via dust supplies is correspondingly low (Boyle, 1997). It is also argued that a fixed Fe:C Redfield ratio is unrealistic because the assimilation, storage and use of Fe enables the Fe:C ratio to be adjusted over a wide range within and between different phytoplankton species (Sunda and Huntsman, 1995b; Sunda, 1997) (see Table 10).

6.1.2. *The biological availability of iron*

Iron has a solution chemistry that is dominated (at pH 8) by extensive hydrolysis, which makes it prone to rapid removal by oxyhydroxide colloid formation and effective scavenging onto falling particles. Solubility estimates (Millero *et al.*, 1995; Sunda and Huntsman, 1995b; Zhu *et al.*, 1992) range from 6.6×10^{-10} M to 10^{-8} M at pH 8.2 and 25°C. This will make the availability of iron very patchy and uncertain if the primary supply by dust particles is episodic. The colloidal iron can be slowly resolubilized (at rates up to 12% per day) (Barbeau and Moffett, 2000) by photochemical action in the near-surface layers. Direct uptake of inorganic iron must involve these soluble hydroxide complexes. The average total iron concentration in the surface layers of the ocean (Johnson *et al.*, 1997a) is 7×10^{-11} M. Thermodynamic calculations (Hudson and Morel, 1990; Rue and Bruland, 1995) indicate that the ratio of dissolved inorganic species to free Fe^{3+} at pH 8 is 10^{11}. The concentration of Fe^{3+} is $<10^{-22}$ M – or just a handful of molecules per litre (Rue and Bruland, 1995)! Alternative strategies are therefore required to gain access to the small and transient total iron reservoir (Figure 8) (Hutchins, 1995; Wells *et al.*, 1995).

The presence of organic complexes increases the solubility of iron (Kuma *et al.*, 1996) and greatly reduces the opportunity for removal of the iron by particle scavenging. It has been observed that phytoplankton may produce excess ligand in response to an influx of iron (Witter *et al.*, 2000), thereby making it accessible for longer periods. The consequence is that the total iron reservoir is stabilized, but has the accessibility of the iron been improved? At the average total levels reported for the euphotic zone (Johnson *et al.*, 1997a) the concentration of dissolved iron not complexed by organic matter is $<10^{-11}$ M, which still represents a very low concentration of available metal.

If the complexing ligand is a siderophore then the formation of the complex will increase the availability for phytoplankton (usually cyanobacteria) that can transport the complex directly across the cell membrane (Wu and Luther, 1995). Little is known about the factors that

Figure 8 The intervention of biology in regulating the availability of iron. Plankton release organic ligands that help to stabilize the dissolved reservoir of iron and make it susceptible to photoreduction thereby releasing Fe (II). Similar cycles are maintained for manganese and copper.

control the rate of assimilation of siderophore-complexed iron. The cyanobacterium *Synechococcus* is known to produce siderophores in low iron media. It is abundant in most marine environments (Olsen *et al.*, 1990) and has the ability to take up a number of different siderophore complexes (Wilhelm and Trick, 1994). Experimental studies suggest that diatoms and dinoflagellates are able to assimilate porphyrin-complexed Fe (III) (Hutchins *et al.*, 1999b). *Emiliania huxleyi* cells have also been found to release iron-complexing ligands in response to iron additions (Boye and van den Berg, 2000). Siderophore release is costly to the cells and only occurs under iron-limited conditions. This tactic is beneficial only when the cell density of phytoplankton is sufficiently high. Such a critical density can be surpassed by cyanobacteria but is never reached by diatoms (Völker and Wolf-Gladrow, 1999). The cyanobacteria may therefore be practising a form of chemical warfare, trapping and assimilating the available iron at the expense of less versatile eukaryotic competitors. This possibility is reinforced by studies of the impact of the fungal siderophore 'Desferal' on natural populations of coastal phytoplankton (Hutchins *et al.*, 1999a; Wells, 1999). The siderophore effectively stopped the uptake of iron by eukaryotic phytoplankton. However, it did not directly affect the uptake of

Mn, Co or Zn (Wells, 1999). For the cyanobacteria themselves the release of siderophores is an act of cooperative altruism. Diatoms are themselves capable of waging a kind of chemical warfare against copepod grazers by releasing aldehydes, which arrest embryonic development (Miralto *et al.*, 1999).

Another way in which the organic complexation of Fe (III) may enhance its availability is by making it more susceptible to photoreduction to Fe (II). Ferrous iron is less strongly complexed than ferric iron, is kinetically more labile (Table 5) and is the form in which the ancestral phytoplankton will have encountered iron. The potential for the generation of the more accessible Fe (II) in surface waters has raised considerable interest (Wells and Mayer, 1991; Kuma *et al.*, 1992; Johnson *et al.*, 1994; Voelker and Sedlak, 1995). There is evidence that Fe (III) can be reduced to Fe (II) via the photo-oxidation of Fe (III) complexing organic matter near the sea surface (Kuma *et al.*, 1992; Miller *et al.*, 1995), and it is likely that a significant proportion of the soluble iron delivered in acid rain is present as Fe (II). The photoreduction of Fe (III)-organic complexes at oxide surfaces also provides a possible route (Waite and Morel, 1984). The photoreduction rate has been reported to be low (10^{-4}–$10^{-2}\,h^{-1}$) compared with the oxidation rate ($20\,h^{-1}$) (Morel *et al.*, 1991), but this imbalance may be favourably influenced if the reduced Fe (II) remains organically complexed. Ferrous iron released in this way can be taken up by diatoms (Rich and Morel, 1990; Sunda and Huntsman, 1997). These photoreduction mechanisms will be most effective in tropical regions where the mid-day irradiance can reach $100\,mW\,cm^{-2}$. Direct measurements of Fe (II) by ligand competition techniques have shown a diurnal pattern of Fe (II) availability in such regions (O'Sullivan *et al.*, 1991). These processes will not be active throughout the euphotic zone because photochemistry is driven primarily by UVB radiation, which has a limited penetration in the oceans.

The photoreduction must be quite closely coupled with the uptake mechanism because of the rapid oxidation rate of Fe (II) to Fe (III) in surface waters, with the lifetime of Fe (II) being measured in minutes to hours. Photochemical processes also continually produce O_2^- and H_2O_2, which are potent oxidizing agents (Moffett and Zika, 1987). This problem can be circumvented by biological reduction at cell surfaces, or by reductases within the membranes, which have been suggested as efficient iron acquisition mechanisms for diatoms (Völker and Wolf-Gladrow, 1999). The fairly rapid oxidation and reduction kinetics involved suggest that there will be considerable recycling of the iron in the surface waters in a manner reminiscent of the very effective re-utilization of NH_4^+ in the microbial loop in oligotrophic regions (Dugdale and Goering, 1967).

The colloidal iron phases may also be accessible to phytoplankton cells (Barbeau and Moffett, 2000). These cells may bind small particles with an active surface (Hutchins *et al.*, 1993) and participate in the general aggregation process. The literature has been reviewed by Hutchins (1995) who suggests that iron-limited cells can internalize this adsorbed iron quite efficiently. A photosynthetic, or more strictly mixotrophic (Sanders, 1991), flagellate from the Pacific (*Ochromonas* sp.) has been shown in culture to obtain Fe by ingesting marine bacteria (Raven, 1997; Maranger *et al.*, 1998). This is a highly efficient process and 30% of the ingested iron is assimilated. The culture experiments, when extrapolated, suggest that this route could supply up to 50% of the total Fe uptake by the entire autotrophic community (Hutchins *et al.*, 1993). Chrysophyceae, Prymnesiophyceae and Dinophyceae generally dominate eukaryotic blooms in iron-limited regions. In the equatorial Pacific up to 50% of the protists are mixotrophic and it is possible that such organisms can also mobilize the colloidal and detrital iron pools (Rich and Morel, 1990; Barbeau and Moffett, 2000).

Trichodesmium species (Falkowski and Woodhead, 1992) are the most widely distributed nitrogen-fixing cyanobacteria in oligotrophic regions. (Capone *et al.*, 1997) The transport of iron in airborne dust is crucial here and *Trichodesmium* species have developed a mobile colonial lifestyle that enables them to trap and solubilize dust particles (Reuter *et al.*, 1992). The colonies are also able to migrate vertically in the water to optimize their catching efficiency and the utilization of light. These species show a preference for warm highly stratified waters (Walsby, 1992) and they are relatively abundant in the iron-rich waters of the Arabian and Caribbean Seas and the Indian Ocean, and relatively less abundant in the subtropical eastern Pacific (Falkowski and Raven, 1997). The ability of these and other prokaryotes to release iron-specific siderophores also enables them to sequester much of the dissolved or colloidal iron that is available.

6.1.3. *The uptake of iron and the control of primary production*

Phytoplankton cells require more moles of iron per mole of carbon fixed than any other trace metal (Table 6). Geider and La Roche (1994) have reviewed the uses of iron within the cell. Primary productivity is limited by iron (Johnson *et al.*, 1997a) when total levels fall below 2×10^{-10} M. Iron therefore has the potential to limit phytoplankton growth (Hart, 1934; Harvey, 1937a, b; Martin and Fitzwater, 1988; Martin and Gordon, 1988; Martin *et al.*, 1989, 1990) especially in HNLC (high nutrient, low chlorophyll) regions (Martin *et al.*, 1991) that constitute 20% of the

ocean surface. The N:P ratios of phytoplankton under iron stress are significantly lower than under iron-replete conditions because iron deficiency impairs nitrate assimilation (de Baar *et al.*, 1997).

Experimental studies with cultures have shown that the coastal (neritic) phytoplankton require much larger quantities of iron per mole of carbon fixed than do open ocean species (Brand *et al.*, 1983, 1991; Sunda *et al.*, 1991; Sunda and Huntsman, 1995b). Oceanic species are also generally smaller and have slower growth rates. The reduction in size increases acquisition rates relative to growth since transport rates are proportional to surface area (i.e. to r^2), whereas requirements are crudely proportional to volume (i.e. to r^3). Species from both environments were found to be taking up iron at rates close to the diffusion limits (Table 7). Oceanic species also have substantially lower iron quotas (Q) than coastal species (see Table 10) and can maintain near maximal growth rates at these very low levels of cellular iron (Price and Morel, 1996). Cyanobacteria have much higher cellular iron requirements than eukaryotes because of the high PSI:PSII ratios built into their photosynthetic apparatus (Raven *et al.*, 1999). The high efficiency with which oceanic phytoplankton use iron can be attributed in part to the progressive substitution of Fe wherever possible. The replacement of ferrodoxin in PSII with flavodoxin has been used as a rapid diagnostic for iron limitation in phytoplankton (La Roche *et al.*, 1996). The replacement of iron-containing cytochrome c_6 by copper-based plastocyanin for electron transfers between cytochrome c_6/b_f and PSI has already been remarked upon (Raven *et al.*, 1999). There is good evidence that the internal iron content of phytoplankton cells is actively regulated to ensure optimum performance in the face of varying external concentrations (Harrison and Morel, 1986).

Studies of iron limitation have focused initially on HNLC conditions that occur in three areas of the world ocean:

A. the subantarctic circumpolar waters in the Southern Ocean
B. the eastern equatorial Pacific
C. the Gulf of Alaska in the subarctic Pacific.

HNLC conditions are found in 40% of all surface waters in the Pacific basin (Reid, 1962). The chlorophyll *a* levels here are typically 0.3–0.4 $\mu g\, l^{-1}$ and all three areas receive nutrient rich waters via upwelling. In the high latitude cases, there is also strong seasonal deep mixing (Wells *et al.*, 1995). They are also areas remote from land that receive little in the way of airborne dust (Duce and Tindale, 1991). The productivity of these areas is far below that anticipated at first sight from the standing stock of nutrients. In general small species of phytoplankton ($<5\,\mu m$) dominate in these areas, including a significant proportion of cyanobacteria. Large

diatoms are usually rare, although small diatoms can dominate in some regions of the Southern Ocean. The cells in HNLC areas exhibit low specific uptake rates for nitrate, indicating that they are not performing at their optimum (Dugdale and Wilkerson, 1991). Conditions in the eastern equatorial Pacific are particularly conducive to phytoplankton growth since there are high, year-round light levels and the water column is periodically stratified, permanent stratification being prevented by frequent storm events. However, the chlorophyll levels are only twice as high as the adjacent oligotrophic areas while the nutrient levels are a thousand times higher (Eppley *et al.*, 1992).

A variety of hypotheses have been put forward to explain these anomalous regions (Cullen, 1991) including: the control of phytoplankton biomass by grazing (Chavez *et al.*, 1991; Frost, 1991; Miller *et al.*, 1991); low light availability because of high latitude (areas A and C, above) and deep mixing (Miller *et al.*, 1991; Mitchell *et al.*, 1991); and the limitation of growth by low iron levels (Martin and Fitzwater, 1988; Martin *et al.*, 1990, 1991).

This review will focus on the iron limitation hypothesis (Falkowski, 1995; Fitzwater *et al.*, 1996). Nonetheless, it should be noted that Platt has recently suggested that the lack of iron may not be a sufficient, or even a necessary condition for the limitation of productivity in HLNC regions (unpublished observations). Rather, he has shown that light may be limiting, since the intermittent mixing down of phytoplankton below the euphotic zone in these regions prevents them from utilizing all the nutrients available. The addition of iron would improve their efficiency but would not allow them to exhaust the nutrient supply. In the Southern Ocean the strong winds throughout the year exert a powerful control on phytoplankton productivity (de Baar and Boyd, 1998). Model studies of co-limitation of nitrate utilization by both iron and light availability (Armstrong, 1999) indicate the importance of cell size in predicting the response of phytoplankton communities to an influx of aeolian iron. Detailed studies in the Pacific (Lindley *et al.*, 1995) have revealed a spectrum of dependence of phytoplankton growth on the availability of iron (see also de Baar and Boyd, 2000).

It is interesting to note that in some HNLC regions the ratio of the total Fe transported upwards by upwelling to the total carbon exported downwards in new production (Watson, 2001) is close to the Fe:C values observed for phytoplankton cell quotas for iron (Table 12: compare Table 10). However, the availability of iron in these regions is patchy (de Baar *et al.*, 1995).

Iron limitation is more general than was first anticipated. It is widespread in the Pacific (Coale, 1991; Nakayama *et al.*, 1995) and has been demonstrated in the Southern Ocean (de Baar *et al.*, 1990; Buma

Table 12 Calculated Fe:C ratios in HNLC regions.[a]

Region	Atmospheric Fe input[b]	Upwelling Fe flux[b]	NO_3^- at surface (μM)	Calculated new production (Gt C yr^{-1})	Calculated Fe:C[c]
Equatorial Pacific	2–100	280	8	1.87	1.8
Antarctic	0.14–6.8	480	25	0.63	9.1
SubArctic Pacific	7.8–390	86	12	0.35	2.9

[a]Compiled from data given in Watson (2001).
[b]10^6 mol yr^{-1}.
[c]μmol Fe per mol C, using upwelling Fe supply.

et al., 1991). However, it is by no means restricted to HNLC regions and it is even observed in coastal waters (Hutchins *et al.*, 1998). Most of the studies of iron limitation have depended on the use of bottle experiments where nitrate uptake and photosynthesis rates have been stimulated by the addition of carefully controlled nanomolar aliquots of iron to indigenous plankton samples incubated under scrupulously clean conditions (Martin *et al.*, 1989, 1991, 1994; de Baar *et al.*, 1990; Buma *et al.*, 1991). Such experiments are open to criticism because of the potential for additional bacterial growth on the bottle walls, and because additional growth might be triggered by the removal of grazing animals.

More direct, and dramatic results have been obtained by enriching large areas (100 km^2) of the ocean with carefully metered additions of iron (Martin *et al.*, 1994; Wells, 1994; Coale *et al.*, 1996a, b) using a tracer technique developed by Watson (Watson and Ledwell, 1988; Watson *et al.*, 1991). In the IRONEX series of experiments in the equatorial Pacific HNLC region to the west of the Galapagos Islands, the iron was released as a strongly acid ferrous sulphate solution mixed with a sulphur hexafluoride tracer (SF_6). The SF_6 tracer enabled the iron-inoculated water to be tagged and quantitative assessments made of the fate of the added iron. A wide range of analyses was carried out (see Table 13) and the data were mapped in real time wherever possible to keep track of events in the iron-enriched water and in the unchanged region surrounding the patch.

The IRONEX I experiment was performed on an area with 11 μM of nitrate and 0.24 $\mu g\,l^{-1}$ of chlorophyll *a*. The initial iron concentrations within the patch after enrichment were as high as 6.2×10^{-9} M, with an average value of 4×10^{-9} M. There were rapid rises in chlorophyll levels (a three-fold increase to 0.65 $\mu g\,l^{-1}$) and primary productivity (a four-fold increase from 10–15 $\mu g\,C\,l^{-1}\,d^{-1}$ to 48 $\mu g\,C\,l^{-1}\,d^{-1}$). Photosynthetic efficiency increased dramatically following improved performance of

photosystem II on the relief of iron stress (Kolber *et al.*, 1994). However, there was little loss of nitrate (<0.2 μM, although NH_4^+ levels did fall) and only a small draw-down of CO_2 (<10 μatm), possibly because the zooplankton grazing pressure remained closely coupled to the phytoplankton growth (Chai *et al.*, 2000). There was an increase in numbers of all phytoplankton groups, except the prochlorophytes. The population of protozoan grazers also increased by 50%. The enriched water mass was subducted to 35 m during the course of the experiment, reducing the possibility of photochemically induced recycling of the iron. The area to the west of the Galapagos, which frequently receives pulses of airborne dust, was also studied and shown to have natural iron enrichment.

In IRONEX II, sequential additions of iron were made in an area where the nitrate concentrations were 10.5 μM and the chlorophyll levels were 0.15–0.2 $\mu g\,l^{-1}$. The results were unequivocal. The mean concentration of Fe in the initial patch was 2×10^{-9} M and this level was maintained by further additions after 3 and 7 days. There was a rapid and dramatic doubling of the photosynthetic efficiency of the picophytoplankton, and an increase in nitrate assimilation rates confirming that the system was iron limited (Behrenfeld *et al.*, 1996). Nitrate concentrations fell to 7.0 μM as a consequence of a diatom bloom (a 20-fold increase in cell numbers) and chlorophyll *a* levels increased 30-fold. Large pennate diatoms were the major beneficiaries. There was a consequent significant draw-down of CO_2 partial pressure from the background level of 510 μatm to 420 μatm within the fertilized patch (Cooper *et al.*, 1996). The addition of extra silica in bottle incubation experiments indicated that the diatom growth could have been greater if silicon had been more readily available in the water column. No limitation by the low ambient levels of zinc was detected.

There was therefore a significant local but transient slowing down of the release of CO_2 from the upwelling water to the atmosphere. The populations of smaller plankton were held in check by efficient protozoan grazing, but there were few metazoan grazers available to take advantage of the diatom bloom. The concentration of DMS in the water increased by a factor of three in the bloom (Turner *et al.*, 1996). These experiments have established that the lack of accessible Fe is an important factor limiting productivity in the equatorial Pacific region.

Greater impacts from iron addition would be expected in the Southern Ocean where the nutrient levels (including silicon) are higher and there is greater potential to sequester the enhanced carbon production into deeper water. An iron release in the Southern Ocean has been performed (UK SOIREE) (Abraham *et al.*, 2000; Boyd *et al.*, 2000) and two more are planned (US IRONEX III, EU CARUSO). In the SOIREE experiment at 61° S 141° E a series of iron releases (on days 1, 4, 6 and 8) was able to stimulate, after an induction period of 5 d, a long-lasting increase in

Table 13 Iron release experiments.

	IRONEX I	IRONEX II	SOIRÉE
Date of release	October 1993	June 1995	February 1999
Site of release	Equatorial Pacific	Equatorial Pacific 3.5° S, 104° W	Antarctic circumpolar current 61°S, 140°E
Nature of release	A single addition of 450 kg Fe over 64 km^2	Repeated additions on days 1, 3, 7	Repeated additions on days 1, 4, 6, 8
Chemical measurements	Major nutrients, CO_2 parameters Particulate organic C, N, P DMS Iron chemistry	As for IRONEX I plus other potential limiting nutrients (Zn, Si) Colloids Metal speciation	As for IRONEX I plus Si Fe speciation Fe uptake kinetics
Phytoplankton biology	Photosynthetic efficiency Chlorophyll, etc. Carbon fixation Analytical flow cytometry (AFC)	As for IRONEX I	As for IRONEX I plus flavodoxin Bacterial abundance
Community structure	AFC	As for IRONEX I plus zooplankton grazing	As for IRONEX II
Key references	Kolber *et al.*, 1994; Martin *et al.*, 1994	Behrenfeld *et al.*, 1996; Coale *et al.*, 1996a	Abraham *et al.*, 2000; Boyd *et al.*, 2000

primary production that could still be detected by satellite some 40 d later (Abraham *et al.*, 2000; Boyd *et al.*, 2000; Chisholm, 2000). Iron levels were enriched up to 3×10^{-9} M compared with background levels of 1×10^{-10} M or less.

Massive blooms of the large diatom *Fragilariopsis kerguelensis* (30–50 μm cell length) began to develop 5 d after the original fertilization, with cell densities of 4.4×10^4 cells per litre by day 12. These heavily armoured cells form chains of a dozen or more individual cells and are "morphologically adapted to minimize grazing" (Verity and Smetacek, 1996). It is surprising to note that Fe (II) (half-life for oxidation 1 h) was the predominant iron species at this time, possibly because of enhanced photo-oxidation of organic complexes encouraged by the long hours of daylight. There was little evidence of the enhanced export of carbon from the surface layers during the 13 days that the bloom was monitored from the research vessel. At the end of the experiment the chlorophyll *a* levels in the water had increased six-fold and the phytoplankton were still not silicon- or iron-limited. Upper ocean levels of dimethylsulphide also increased and the partial pressure of CO_2 and the content of total dissolved inorganic carbon both decreased during the experiment. A significant increase in bacterial production rates followed the rise of the diatom bloom, although their numbers were kept in check by active grazing. For example, ciliate numbers increased four-fold.

The persistence of the bloom has been modelled in some detail (Abraham *et al.*, 2000). Forty days after the initial iron release over an 8 km diameter patch, the bloom had stretched into an omega-shaped ribbon some 150 km long and up to 10 km wide. A hydrodynamic analysis indicated that on day 13 only 35% of the phytoplankton production was being stirred out of the original patch and smaller amounts were being lost through zooplankton grazing (<5%) and vertical sinking (<10%). The stirring would introduce fresh nutrients (especially silicon) into the patch but it would also dilute the added iron. The iron levels were estimated to be 2×10^{-10} M (twice the background level) on the day of the final satellite image. The persistence of the bloom therefore appears to rest on:

1. the biological processing of the added iron into an organically bound form that was not rapidly stripped from solution and
2. a favourable balance of hydrodynamic forces that effectively stirred the patch without diluting it to the point where the iron enhancement was no longer effective.

Proponents of the sequestering of atmospheric carbon dioxide by iron fertilization of the ocean should not draw comfort from this experiment, however. There was no evidence of the enhanced export of particulate carbon to deeper waters (Chisholm, 2000; Watson *et al.*, 2000).

6.1.4. *Interactions with macronutrient cycles*

The biochemistry of iron is closely linked to that of nitrogen (Geider and La Roche, 1994; Price *et al.*, 1995) because Fe is involved in the assimilation of all forms of inorganic nitrogen (NO_3^-, NO_2^-, N_2), with the exception of NH_4^+. Clear effects of iron stress on nitrate metabolism observed in phytoplankton (Timmermans *et al.*, 1994, 1998) have been attributed to its involvement in the generation of reducing power, its role as a co-factor in the nitrate reductase. This provides a direct mechanistic link between Fe concentrations and new production (Table 12).

This observation relates to the suggestion that the widespread deficit of nitrate in the ocean surface results from chronic nitrogen losses to deeper water through export in particulate matter, combined with iron limitation of N_2 fixers in the upper ocean (Howarth *et al.*, 1988; Raven, 1988; Falkowski, 1997). Falkowski considers that the evolution of denitrifiers preceded nitrogen fixation and that the relatively small size of the current marine nitrogen-fixation flux is compatible with its role as the long-term controller of ocean nitrate content (Mancinelli and McKay, 1988). In contrast, Tyrrell's model (Tyrrell, 1999) produces a nitrate deficit without assuming trace metal effects. Lenton (1998b) has also shown that a regulatory system of this kind can produce Redfield ratios between the macronutrients and oxygen in a model ocean without recourse to iron limitation.

Toggweiler (1999) has suggested that the potential limitation of nitrogen fixation by the shortage of available iron (Falkowski, 1997; Karl *et al.*, 1997; not modelled by Tyrrell, 1999) could make the nitrogen balance dependent upon iron supply. This view is supported by Cullen (1999). A sustained input of iron (e.g. from enhanced atmospheric dust supplies) could increase nitrogen fixation to the point where all available phosphorus is stripped from the upper ocean, leading to curtailed phytoplankton production. The phytoplankton in a nitrogen-replete ocean would withdraw an additional $6 \times 10^{17}\,\mathrm{g\,C\,yr^{-1}}$. This may be compared with an estimate of $1.4 \times 10^{17}\,\mathrm{g\,C\,yr^{-1}}$ if the direct fertilization of HNLC zones with Fe enabled all the phosphorus to be utilized. To set this in context, $8 \times 10^{17}\,\mathrm{g\,C\,yr^{-1}}$ would need to be sequestered to pull atmospheric CO_2 partial pressures down from 280 ppm to 190 ppm, as was experienced in the last interglacial to glacial transition. A recent comparative study (Wu *et al.*, 2000) demonstrates phosphorus depletion fuelled by enhanced nitrogen-fixation in the iron-rich North Atlantic, in contrast to nitrogen depletion in the presence of excess phosphorus in the iron-depleted North Pacific gyre.

Toggweiler suggests that iron complexation in deep water may be considered to regulate the availability of iron in upwelling waters (Johnson *et al.*, 1977a).

> Indeed it may turn out that organisms alter the cycling of Fe in such a way that in the ocean it is regulated by the river input of phosphate, just like nitrate. (Toggweiler, 1999)

In equatorial Pacific HNLC regions the addition of Fe induces the growth of diatoms, which are best able to take advantage of the large standing stock of nitrate. Their high silicon requirement will put a ceiling on the extent to which the available nitrogen can be assimilated when Fe is added (Dugdale and Wilkerson, 1998). The inter-relationship between the Fe and Si cycles is quite subtle since the Fe addition does not simply permit the assimilation of C and N, but it affects the way in which silica itself is deposited (Smetacek, 1998).

Under Fe-deficient conditions, diatoms reduce the uptake of nitrate and survive with lower cellular levels of nitrogen and phosphorus. However, diatoms from the subarctic Pacific, the high latitude Southern Ocean and the eastern Pacific increase the uptake ratios of Si:N and Si:P under Fe limitation – thereby accelerating the removal of Si from surface waters (Takeda, 1998). In effect, the diatoms rapidly lay down the defensive silica skeleton using low cost inorganic processes. The biomass and composition of the cells grown within these defences will depend upon the availability of nutrients. Diatoms stressed by a lack of iron will therefore deplete the surface waters of Si before N, leading to a secondary limitation by a lack of Si. These cells will have heavier silica shells and will also result in an unusually high export of particulate matter to deeper water, possibly comparable to that recently attributed to giant diatoms (Smetacek, 2000). Similar effects are also noted in coastal upwelling regions where Fe limitation occurs because the continental shelf is narrow (with a correspondingly small sedimentary source of Fe) and there are no rivers draining into the shelf area (further decreasing iron inputs) (Hutchins and Bruland, 1998).

The shells of Fe-limited diatoms are thicker and therefore sink faster and lose less Si by dissolution in the water column; thus they will be more effectively preserved in the sediments. From this perspective, the sedimentary records of C and Si deposition in the glacial Southern Ocean (Kumar *et al.*, 1995) are consistent with the idea that changes in productivity and thus in the draw-down of atmospheric CO_2 during the last glaciation were stimulated by changes in Fe inputs from atmospheric dust (Martin, 1990). But deposition from regions where production is enhanced

by Fe (dust) additions would not rain down proportionately more silica – making the interpretation of the silica deposition more complex (Boyle, 1998).

The distribution of Si, N and P within the oceans is therefore modulated by the availability of iron. Si is removed more rapidly to the deep ocean than N and P when the Fe supply is limited over large areas (the contemporary position). The vertical and inter-ocean contrasts between Si and N and P may therefore have been less during glacial periods when Fe was more widely available because of the enhanced transport of atmospheric dust (Boyle, 1998). However, the dust also transported significant quantities of silicon to the oceans (Trégeur and Pondaven, 2000) and so the palaeo-oceanographic evidence does not support unequivocally the hypothesis of iron limitation of global marine primary production (Loubère, 2000; Paytan, 2000).

6.1.5. *The community effects of iron*

Iron affects community structure by influencing the size spectra of the predominant primary producers (Hutchins, 1995). This in turn controls the degree of influence of the microbial loop and the rate at which the ecosystem exports particulate matter into deeper water. Conversely, the community structure affects the degree to which iron is recycled in the euphotic zone and can enhance the extent to which the total iron pool is utilized by increasing the availability of the iron that is present. The dynamics of such community interactions have been considered for the macronutrient elements by Elser and Urabe (1999) who show that the "... stoichiometry of nutrient release is a feedback mechanism linking grazer dynamics and algal nutritional status".

The iron enrichment experiments have shown that supplying iron to impoverished phytoplankton communities causes a temporary shift in dominance from small cells to larger diatoms (Martin *et al.*, 1989; Buma *et al.*, 1991; Price *et al.*, 1994). The cyanobacteria, which usually predominate in nutrient-limited areas, are aided by their large surface to volume ratios and by special mechanisms for optimizing the uptake of iron (e.g. siderophore release by *Synechococcus* and particle solubilization by *Trichodesmium*), but they are hindered by their high iron requirements. The dominant group in both oligotrophic and HNLC regions is *Prochlorococcus* (Chavez *et al.*, 1991), but little is known about its iron requirements or acquisition strategy. This was the only group whose numbers did not increase in the IRONEX I experiment (Martin *et al.*, 1994).

Figure 9 The involvement of the microbial community in the cycling of iron. The figures represent the budget per litre in the top 80 m of the water column (Price and Morel, 1996). Fluxes shown are in units of $10^{-12}\,\mathrm{M\,d^{-1}}$. The areas of the circles representing the reservoirs are proportional to the percentage contribution to a total iron concentration of $5.6 \times 10^{-10}\,\mathrm{M}$. The measurements were made at ocean station Papa in the north-east Pacific (50°N, 145°W).

An analysis of iron partitioning in an oceanic plankton community (Price and Morel, 1996) (Figure 9) indicates the tight coupling in the iron utilization and excretion of the various trophic levels. The organisms contain a pool of iron approximately equivalent in size to the dissolved iron pool, representing only a small fraction (<10%) of the total iron. Half of the iron is held in the biological detritus. Most (85%) of the iron requirements of the producers are met by regenerated iron released from the higher trophic levels (Hutchins *et al.*, 1993, 1995). The protozoans provide 90% of this recycled supply by processing the producers and the detrital material in equal measure. These organisms are also capable of releasing soluble iron from colloidal ferrihydrite. Although the rates are significantly less than those achieved by photolysis in the near-surface layers, the overall conversion flux for the biological process dominates because it is active throughout the euphotic zone (Barbeau and Moffett, 2000).

Figure 10 The cycling of iron by the microbial loop in the subarctic Pacific. The values quoted by Kirchman (1996) for the whole water column have been converted to the values per litre (assuming a water volume of $100\,m^3$) to put them on the same scale as those used by Price and Morel (1996) and represented in Figure 9. Fluxes are therefore given in units of $10^{-12}\,M\,d^{-1}$ and reservoir sizes in units of 10^{-12} M. Drawn from data compiled by Kirchman (1996) and Tortell *et al.* (1996).

There is evidence from culture experiments on samples from the subarctic Pacific that heterotrophic bacteria may account for 50% of the biomass and 20–45% of the iron uptake in these ecosystems (Kirchman, 1996; Tortell *et al.*, 1996). There is direct competition between these bacteria and phytoplankton for the scarce iron resources. Indeed there is evidence of the direct stimulation of growth by iron in similar bacteria sampled from the Antarctic (Pakulski *et al.*, 1996). The growth of these bacteria will also be limited by the availability of a suitable dissolved organic carbon substrate. This introduces another component of the ecosystem cycling of iron (see Figure 10) which has been appropriately called the "microbial ferrous wheel" (Kirchman, 1996). In this system, as in the model of Price and Morel (1996), the grazers return to the dissolved pool up to two thirds of the iron that they assimilate. This efficient

community turnover has also been noted for the metabolism of Zn and Mn in experimental microcosms (Hutchins and Bruland, 1994) and in coastal communities (Hutchins and Bruland, 1995).

6.2. Manganese

6.2.1. *Distribution of manganese in the oceans*

Manganese is primarily present as Mn^{2+} in sea water and it exhibits complicated and time-varying profiles in the ocean related to the multiple sources of this element (Landing and Bruland, 1980; Martin and Knauer, 1980; Yeats and Bewers, 1985; Burton and Statham, 1988; Jickells and Burton, 1988; Saager *et al.*, 1989). The hydrothermal flux for Mn^{2+} is greater than the input from the rivers (Appendix II). In addition manganese, like iron, is transported to the oceans in airborne dust (Duce *et al.*, 1991; Nakayama *et al.*, 1995). Manganese is scavenged from the water column at all depths. In the case of manganese the scavenging is oxidative rather than hydrolytic:

$$Mn^{2+} + 2H_2O \leftrightarrow MnO_2 + 4H^+ + 2e^-$$

and it is catalysed (Stumm and Morgan, 1981; Grill, 1982) by the presence of solid MnO_2. The deposits of manganese nodules on large areas of the ocean floor are a testament to this inexorable process (Glasby and Read, 1976). The first order residence time for Mn^{2+} in the water column relative to this removal process is 50 yr (Weiss, 1977). This high rate of scavenging is responsible for the variability of the manganese concentration profiles and their dependence on the strength of local inputs.

The oxidation process is readily reversible and it is driven in both directions by different populations of bacteria (Grill, 1982; Cowen and Bruland, 1985). At low oxygen partial pressures within the oxygen minimum zone just below the thermocline there is evidence for the regeneration of Mn^{2+} from particulate manganese in productive regions (Klinkhammer and Bender, 1980; Johnson *et al.*, 1996). There is also a large but unquantified flux from organic-rich sediments, particularly on the continental shelf and slope (Martin *et al.*, 1985; Heggie *et al.*, 1987). In general, vertical profiles of Mn_T concentration have a surface maximum with declining concentration with depth. This gradual decline may be punctuated by maxima arising from the horizontal transport of Mn^{2+} from shelf slope sediments or from hydrothermal inputs (Weiss, 1977). Manganese mapping is in fact an established procedure for locating deep sea hydrothermal vents (Burton and Statham, 1988). There is a decline in deep

water concentrations on moving from the Atlantic to the Pacific, discounting deep areas of high concentration resulting from hydrothermal inputs.

6.2.2. *Biological availability of manganese and impact on primary production*

The steady oxidation of Mn^{2+} by inorganic and microbial processes is counterbalanced by a photoreduction of MnO_2 to Mn^{2+}, probably via the photo-oxidation of organic matter adsorbed at the surface of suspended MnO_2 particles (Sunda *et al.*, 1983; Sunda and Huntsman, 1988, 1990a). This process is also accompanied by a photoinhibition of the microbially mediated oxidation of Mn^{2+}. Together, these processes impart a diurnal cycle on the concentration of available manganese in the surface layers of the ocean (Sunda and Huntsman, 1990a). There is no evidence for significant organic complexing of Mn^{2+} in the surface waters of the ocean and there is only relatively weak inorganic complexation (Turner *et al.*, 1981; Byrne *et al.*, 1988). Therefore, although the concentrations of available Mn^{2+} are low ($1–2 \times 10^{-9}$ M), they are several orders of magnitude higher than the available iron concentrations. Since the exchange kinetics for Mn^{2+} exchange are rapid (Table 5) and cellular demand for Mn is much lower than for Fe, it would not be expected at first sight to act as a limiting nutrient element.

However, culture experiments on the regulation of growth by the availability of manganese suggest that limitation could occur in upwelling regions isolated from aeolian and terrestrial sources, since the deep waters are depleted of Mn^{2+} by persistent oxidative scavenging (Brand *et al.*, 1983). This suggestion is supported by work on manganese limitation in culture experiments with chlorophyte species *Chlamydomonas* (Sunda and Huntsman, 1985) and diatom species *Thalassiosira* (Sunda and Huntsman, 1986). There is active regulation of internal manganese concentrations by phytoplankton cells in the face of over a 100-fold change in external concentrations (Sunda and Huntsman, 1985). This is achieved by modulating the uptake rate through the active production of uptake ligands at the cell surface. The interaction between the cellular regulation of manganese concentration and the uptake of other metals has been considered earlier (Section 4.2.4). The interactions with copper are most likely to generate significant manganese deficiency in upwelling waters (Sunda *et al.*, 1981; Sunda and Huntsman, 1983; Murphy *et al.*, 1984; Sunda, 1989; Bruland *et al.*, 1991).

An interesting and potentially significant link has been made between the chemistry of manganese and the formation of molecular nitrogen (Luther *et al.*, 1997). In the presence of molecular oxygen and MnO_2,

the catalytic oxidation of organic nitrogen and ammonium to molecular nitrogen is possible without biological mediation. In anoxic conditions the direct reduction of nitrate to molecular nitrogen by Mn^{2+} has also been observed. It is estimated that over three quarters of the ammonium generated from organic matter may be converted to nitrogen via the catalytic process involving MnO_2.

6.3. Copper

6.3.1. *Distribution of copper in the oceans*

The distribution of copper in the world ocean does not fit neatly into the categories of vertical profiles described in Section 2.1.3. While total Cu levels (Cu_T) do show depletion in the surface layers relative to the deep water (e.g. 0.5×10^{-9} M at the surface, 5×10^{-9} M at 5000 m in the North Pacific) (Coale and Bruland, 1990) – the concentration profile is almost linear with depth. This is considered to result from the modification of a typical nutrient profile by Cu scavenging onto particulate matter at all depths (Boyle *et al.*, 1977; Nolting *et al.*, 1991). This tendency is predicted from the scavenging indices (Whitfield and Turner, 1987; Clegg and Sarmiento, 1989) and results in a lifetime of only 1000–2000 yr for copper relative to removal by particles (Boyle *et al.*, 1977). Cu_T concentrations in the North Atlantic (1.2×10^{-9} M) are somewhat higher at the surface than in the Pacific and lower at depth (2×10^{-9} M) (Collier and Edmond, 1984) and higher again in the Southern Ocean (Nolting *et al.*, 1991) (surface 2×10^{-9} M, deep 3×10^{-9} M). Here the profiles are variable, indicating local inputs from coastal or sediment sources. A strong influence of aeolian sources is noted on the distribution of Cu_T concentration in the Indian Ocean with surface maxima ranging from 2–4 $\times 10^{-9}$ M (Saager *et al.*, 1992). Deep water scavenging is also noted here with Cu_T concentrations falling to a minimum of 5×10^{-8} M to 1×10^{-9} M at 500 m. However, the concentrations rise again below this depth to 2–3 $\times 10^{-9}$ M in deeper water, with increased concentrations (up to 8×10^{-9} M) near the sediment surface, suggesting sediment remobilization (Heggie *et al.*, 1987). A similar pattern has been observed for manganese (Saager *et al.*, 1989). This is consistent with the remobilization of these elements in suboxic and anoxic sediments (see Table 3).

Cu_T concentrations have been observed to correlate quite well with those of silicon (Appendix IIIA). The results are not as consistent nor as universal as those recorded for N versus P, and there are areas where the correlations break down altogether (Orren and Monteiro, 1985). Although

some interaction has been observed between Cu (and Zn) concentrations and the assimilation of Si by diatoms (Reuter and Morel, 1981), the physiological link is not really strong enough to explain the correlations observed. An alternative explanation is provided by the possibility of Cu scavenging by the plankton and the detrital particles themselves (Saager *et al.*, 1992). Suffice it to say that the correlations do exist and they show some tendency for the Cu:Si ratio to decrease on moving from the Atlantic to the Pacific as Si builds up in the deep water. There is a less marked increase in Cu_T in deep water on moving from the Atlantic to the Pacific. This confirms a large degree of biological control on the Cu_T concentrations throughout the world ocean.

6.3.2. *Biological availability of copper and impact on primary production*

The influence of biology on the marine chemistry of copper is even more marked when its chemical speciation is considered. The observed Cu_T concentrations in sea water are close to or exceed the toxicity limits for cyanobacteria (Sunda and Gillespie, 1979; Sunda and Ferguson, 1983; Sunda *et al.*, 1984; Hering *et al.*, 1987) and eukaryotic phytoplankton (Morel *et al.*, 1978; Brand *et al.*, 1986; Sunda and Huntsman, 1995c). Prokaryotes are significantly more susceptible to Cu toxicity than eukaryotes, with the sensitivity increasing in the sequence diatom-dinoflagellate-coccolithophore-*Synechococcus*. This is consistent with the evolution of the prokaryotic requirements in an anoxic ocean where Cu was held at very low levels by CuS formation (Brand *et al.*, 1986). Cu is much more readily available in oxygenated sea water (Turner *et al.*, 1981). The sensitivity to copper toxicity is such that serious difficulties arose with the incubation methods used for the determination of primary productivity for natural phytoplankton samples, as the ^{14}C reagents used contained traces of copper contamination.

In the surface waters of the ocean, the biologically available concentration of copper is reduced dramatically by the formation of strong organic complexes (Coale and Bruland, 1988) that bind >99% of the Cu_T. The stability constant for the formation of the complex ($10^{-13}\,M^{-1}$) and the covariation of the ligand concentration with Cu_T appear to buffer the free Cu^{2+} concentration at 10^{-13} M in coastal and near-surface waters (Coale and Bruland, 1988, 1990; Moffett *et al.*, 1990; Sunda and Huntsman, 1990b). The complex appears to be broken down by bacterial activity in the deeper water so that the uncomplexed copper concentration can increase by a factor of 2000 at 300 m depth. This has significant implications for the suitability of upwelling water for promoting phytoplankton growth, since Cu^{2+} concentrations in excess of 10×10^{-12} M are toxic to

eukaryotic phytoplankton (Sunda and Huntsman, 1995c), and prokaryotic species are significantly more sensitive. There is some evidence that the Cu (II)-organic complexes are prone to photochemical attack, with the subsequent reduction of Cu (II) to Cu (I) (Moffett and Zika, 1983). Cu (I) forms strong chloro-complexes and could be stabilized in sea water (Turner *et al.*, 1981; Nelson and Mantoura, 1984; Turner and Whitfield, 1987). The implications of this redox reaction for the biological uptake of copper are unclear (Bruland *et al.*, 1991).

Culture studies (McKnight and Morel, 1979; Gerringa *et al.*, 1995) and observations in the field indicate that the complexing ligands have a biological origin (Coale and Bruland, 1988, 1990). The strongest evidence comes from studies of the cyanobacterium *Synechococcus* (Moffett *et al.*, 1990), suggesting that this is the main source of the ligands in the open ocean. *Synechococcus* is widely distributed and can contribute up to 50% of the primary production in some open ocean regions (Iturriaga and Marra, 1988). This ability to release copper-complexing ligands is not necessarily confined to prokaryotes, as there is also evidence that the coccolithophorid *Emiliania huxleyi* can do this in response to copper additions (Leal *et al.*, 1999). Culture studies have recently shown (Croot *et al.*, 2000) that both prokaryotes and eukaryotes are capable of producing Cu-complexing ligands with stability constants of 10^{12}–$10^{14}\,M^{-1}$ (class 1 ligands). Some eukaryotes (notably dinoflagellates) can also produce ligands with stability constants of 10^{9}–$10^{12}\,M^{-1}$ (class 2 ligands). Croot *et al.* (2000) suggest that prokaryotes are the most likely source of the bulk of the class 1 ligands in the open ocean.

Culture studies of three diatoms (*Thalassiosira weissflogii*, *Thalassiosira pseudonana, Thalassiosira oceanica*) and a coccolithophorid (*Emiliania huxleyi*), linked to observations of copper chemistry in the field, have revealed a surprising level of interaction between phytoplankton physiology and the cycle of copper (Sunda and Huntsman, 1995c). The culture experiments indicate that the Cu:C ratios within the cells for all species, except *Thalassiosira weissflogii,* are related by a single curve to the concentration of free Cu^{2+} in the culture medium (Figure 11). The sigmoidal curve indicates active regulation of the copper concentration within the cells (da Silva and Williams, 1991). The absence of this characteristic in *Thalassiosira weissflogii* could indicate a potential role for copper in structuring marine ecosystems.

Although the Cu_T versus Si plots obtained from the deep ocean profiles are not always convincing (Appendix IIIA), strong local correlations have been observed between Cu_T and P within the surface mixed layer above the nutricline (Table 14) (Sunda and Huntsman, 1995c). Good correlations are obtained for remote oceanic regions from a wide geographic range and surprisingly consistent Cu:P ratios can be estimated from these plots.

Figure 11 The regulation of copper accumulation in eukaryotic phytoplankton cells. The cellular Cu:C ratio (μmol mol^{-1}) increases with the free Cu^{2+} concentration in the culture medium on a sigmoidal curve indicating regulation of cell Cu quotas over the concentration range (Sunda and Huntsman, 1995c). (A) *Emiliania huxleyi*, (B) *Thalassiosira oceanica*, (C) *Thalassiosira pseudonana*. The dashed straight line (D) is the regression line for the diatom *Thalassiosira weissflogii*, which does not show regulation of cellular Cu levels. (Redrawn from Sunda and Huntsman, 1995c).

Assuming that the stoichiometry in the water column reflects the uptake of these elements by phytoplankton, Cu:C ratios can be calculated assuming the Redfield ratio C:P = 106 for uptake. A mean value of 4.1 μmol mol^{-1} is obtained for the North Pacific profiles. In this region, the concentration of free Cu^{2+} ranges from 3×10^{-14} M to 2×10^{-13} M with a mean value of 6×10^{-14} M. From the experimental plot (Figure 11) this corresponds to a mean Cu:C ratio of 4.2 μmol mol^{-1}, convincingly close to the value estimated directly from the field data. Furthermore, direct measurements of the Cu:C ratios on net samples of phytoplankton gives values very close to these estimates (Table 14). When this evidence is taken together with the calculations presented earlier on the interactions between Cu and Mn availability in the oceans (Sunda *et al.*, 1981; Sunda and Huntsman, 1983; Bruland *et al.*, 1991), it is clear that the distribution and speciation of copper in the surface layers of the open ocean are controlled directly by biological processes.

Table 14 Correlations between copper and phosphate in natural waters and copper and carbon in phytoplankton cells.[a]

Location	Depth (m)	$\Delta Cu/\Delta PO_4 \times 10^3$	Cu:C $\times 10^6$	Reference
Observed values[b]				
North Pacific				
32.7° N, 145.0° W	0–985	0.44	4.2	Bruland, 1980
39.6° N, 140.8° W	0–780	0.45	4.3	Martin *et al.*, 1989
45.0° N, 142.9° W	0–900	0.43	4.0	Martin *et al.*, 1989
50.0° N, 145.0° W	0–800	0.43	4.0	Martin *et al.*, 1989
North Atlantic				
47° N, 20° W	0–800	0.30	2.8	Martin *et al.*, 1993
Drake Passage				
60.8° S, 63.4° W	30–300	0.68	6.4	Martin *et al.*, 1990
Calculated values[c]				
Equatorial Pacific		0.54	5.1	Collier and Edmond, 1983
S. California and N. Mexico		0.49	4.6	Martin *et al.*, 1976

[a]Reproduced from: Sunda, W. G. and Huntsman, S. A. (1995c). Regulation of copper concentration in the oceanic nutricline by phytoplankton uptake and regeneration cycles. *Limnology and Oceanography* **40**, 132–137, with the permission of the copyright holders, The American Society for Limnology and Oceanography.
[b]Based on depth-dependent variations in dissolved copper versus phosphate in oceanic nutriclines, using a Redfield C:P ratio of 106:1.
[c]Calculated values from natural plankton samples.

6.4 Zinc

6.4.1 *Distribution of zinc in the oceans*

Zinc exhibits vertical profiles characteristic of a recycled (nutrient-like) element with surface depletion and deep water regeneration. In the North Atlantic, surface total zinc (Zn_T) concentrations are around 1×10^{-10} M, with values increasing to 1.5×10^{-9} M at depth (Collier and Edmond, 1984). Similar surface Zn_T concentrations are observed in the North Pacific (Bruland *et al.*, 1978a; Bruland, 1980), but the deep water values are substantially higher, rising to 8×10^{-9} M. Values reported for the Indian Ocean (Saager *et al.*, 1992) show surface values of $1–3 \times 10^{-9}$ M with deep water values rising as high as $9–12 \times 10^{-9}$ M. The increase in deep water Zn_T concentrations on moving from the Atlantic to the Pacific is consistent with the behaviour of the macronutrient elements.

As for copper, the depth profiles resemble those of Si, but the correlations observed for Zn_T are more consistent (Saager *et al.*, 1992).

The slope of the correlation between Zn_T and Si observed in the Indian Ocean (0.05–0.09 mmol mol^{-1}, mean 0.06 mmol mol^{-1}) is similar to that found in the Pacific (range 0.05–0.06 mmol mol^{-1}), but lower than in the Atlantic (0.09–0.17 mmol mol^{-1}). The deep water Zn:Si ratio decreases dramatically between the North and the South Atlantic (Figure 12) (Saager *et al.*, 1992), as a consequence of the rise in Si concentrations. Zn_T correlations with phosphate have also been noted in the surface waters of the Pacific (Sclater *et al.*, 1976; Bruland, 1980) and in the Atlantic (Bruland and Franks, 1983; Yeats and Campbell, 1983; Danielsson *et al.*, 1985).

Figure 12 Redfield ratios for Zn:Si in the world's oceans. Consistent ratios are observed in the deep water for all locations except at 50°N in the North Atlantic (▲) where deep water Si levels are low and Zn concentrations are high because of aeolian input. ■, north-east Pacific; ○, north-west Indian Ocean; ●, Antarctic; △ north-west Atlantic (34°N). Reproduced from: Saager, P. M., de Baar, H. J. W. and Howland, R. J. (1992). Cd, Zn, Ni and Cu in the Indian Ocean. *Deep-Sea Research* **39**, 9–35, with the permission of the copyright holders, Elsevier Science.

6.4.2. *Biological availability of zinc and impact on primary production*

Electrochemical studies (van den Berg, 1985; Bruland, 1989; Donat and Bruland, 1990) have shown that organic complexing agents sequester a large proportion of the Zn_T in the surface layers of the ocean. For example, >98% of the Zn_T is complexed by Zn-specific ligands at depths shallower than 200 m in the North Pacific (Bruland, 1989). The free Zn^{2+} concentration is therefore $<1 \times 10^{-12}$ M in these surface layers. The ligand concentration remains in excess down to 350 m, but the free Zn^{2+} concentration increases 1400-fold from 200 to 600 m. The ligand concentration (1.2×10^{-9} M) is effectively constant throughout the top few hundred metres of the water column.

There appear to be no advantages to the phytoplankton population in trapping the zinc in this way (Bruland *et al.*, 1991) except perhaps to restrict its detrimental effect on the uptake of manganese (Figure 6). There are no immediate solubility constraints on the zinc concentration and it does not undergo redox cycling, so that the benefits conferred by complexation upon iron availability do not apply here. Zinc is an essential element, particularly for eukaryotes and the organic complexation – rather than preventing toxic interactions as was the case with copper – actually reduces the free concentration to potentially limiting levels. Culture experiments (Brand *et al.*, 1983; Sunda and Huntsman, 1992) have shown that neritic species are limited by free Zn^{2+} concentrations $<3.2 \times 10^{-12}$ M, whereas oceanic species are only slightly limited at free concentrations $<3 \times 10^{-13}$ M. This suggests that phytoplankton are adjusting their requirements to match the ambient Zn^{2+} levels rather than vice versa. The ligands themselves may be released inadvertently upon the death of phytoplankton or they may be generated by other components of the planktonic ecosystem (Sunda and Gessner, 1989).

More detailed studies using phytoplankton cultures of coastal (*Thalassiosira weissflogii*, *Thalassiosira pseudonana*) and open ocean (*Thalassiosira oceanica*, *Emiliania huxleyi*) species reveal some interesting facets of zinc uptake (Sunda and Huntsman, 1992). The growth at low Zn^{2+} concentrations was achieved by the oceanic species reducing their internal zinc requirement. At the other end of the scale, the growth of the coastal species *Thalassiosira weissflogii* was restricted at free Zn^{2+} concentrations $> 10^{-7}$ M, whereas the growth of the oceanic coccolithophorid *Emiliania huxleyi* was unaffected. Sigmoidal plots of the Zn:C ratio within the cells against the free Zn^{2+} concentrations of the growth medium (Figure 13) showed very similar behaviour for all species, irrespective of their origins. Kinetic experiments showed that the cells took several hours to adjust to changes in the ambient Zn^{2+} concentrations, which is similar to the rates observed for Mn uptake (Sunda and Huntsman, 1986).

Figure 13 The regulation of zinc levels in eukaryotic phytoplankton. The shaded area shows the envelope of response in cultures of different species of phytoplankton (*Emiliania huxleyi*, *Thalassiosira oceanica*, *Thalassiosira pseudonana*, *Thalassiosira weissflogii*). The symbols represent values observed at three stations in the North Pacific. The free Zn^{2+} values were calculated for these points using observed values of the organic complexation of zinc. (Redrawn from data given by Sunda and Huntsman, 1992.)

The adaptation of oceanic species to low available zinc levels is achieved by lowering the intracellular requirements for zinc. The alternative strategy of improving the efficiency of the Zn uptake mechanism is not available for the diatoms because the system is already running close to the physical diffusion limit for transport to the cell surface (Wolf-Gladrow and Reibesell, 1997; Hudson, 1998) (see Table 7). In such circumstances, element substitutions become important and the ability of Co and Cd to substitute for Zn could be exploited, although their use is restricted by their low total concentrations in sea water.

Localized plots of Zn_T versus phosphate show approximately linear correlations above the nutricline analogous to those observed for copper. From the Zn_T:P ratios obtained from the slopes of these plots, and using a Redfield C:P ratio for uptake (= 106) it is possible to calculate the mean

Zn_T:C ratio in the surface layer. These values relate closely to the ratios observed in the culture studies when they are plotted against the free Zn^{2+} concentration in the ambient sea water (Figure 13).

> Thus, as in the Redfield model for major nutrients, this agreement provides strong evidence that uptake of Zn by phytoplankton is primarily responsible for its removal from surface sea water and directly accounts for distributional patterns for Zn concentrations in the nutricline. (Sunda and Huntsman, 1992).

There is no regulatory role observed or implied here. Phytoplankton are optimizing their performance by making very parsimonious use of the zinc that they can acquire in an environment where the available zinc levels have been depressed by their own exudates.

In this competitive environment, the complexation of zinc with natural organic ligands may make it unavailable for the formation of external carbonic anhydrase (Ellwood and van den Berg, 2000) which catalyses the transport of molecular CO_2 to RubisCo. While Co and Cd can substitute for Zn in this role (Price and Morel, 1990; Lee and Morel, 1995; Lee *et al.*, 1995; Cullen *et al.*, 1999), the extent to which this actually takes place in natural populations is uncertain. Consequently it has been proposed that Zn and CO_2 could be co-limiting in situations where the alkalinity and the pH are high and inadequate mixing reduces the supply of CO_2 from the atmosphere (Riebesell *et al.*, 1993; Morel *et al.*, 1994). The impact is felt most directly by relatively large diatoms that are least efficient at taking up the low concentrations of Zn. Experiments with *Thalassiosira weissflogii* have shown that the uptake of carbon from HCO_3^- uptake is modulated by the Zn concentration. An alternative strategy to maintain photosynthesis at low Zn levels has been revealed by the finding that *Thalassiosira weissflogii* is capable of fixing most of its carbon via C_4 photosynthesis when its cells are under zinc stress (Reinfelder *et al.*, 2000; Riebesell, 2000). C_4 photosynthesis occurs in the cytoplasm and does not require the mediation of RubisCo aided by carbonic anhydrase.

Experiments on coccolithophorids to assess the efficiency of their photosynthetic carbon fixation at low Zn levels would be valuable since they exhibit little or no carbonic anhydrase activity (Sikes and Wheeler, 1982; Quiroga and Gonzalez, 1993) but they can release localized molecular CO_2 supplies by the precipitation of $CaCO_3$. If this enabled coccolithophorids to thrive at low Zn^{2+} concentrations it would increase the rain ratio (molar ratio of carbonate:organic carbon) and decrease the rate of the particle pump for drawing down atmospheric CO_2.

6.5. Cadmium

6.5.1. *Distribution of cadmium in the oceans*

Cadmium is a soft b-type cation and it falls within the same area of the complexation field diagram (Figure 1) as Cu (I) and Hg. Cadmium is a weaker Lewis acid than zinc and unlike copper does not exhibit redox chemistry and so it is far less versatile than either zinc or copper. It is also less abundant than either of these elements. It does however bind more strongly than Zn or Cu (I) to sulphur-based ligands and it is potentially toxic.

Cadmium sulphides are slightly less soluble than those of zinc. The concentrations of cadmium in the oceans would therefore have increased alongside those of zinc when aerobic conditions prevailed. Cadmium can substitute for zinc in a number of enzymes including carbonic anhydrase, in situations where external complexation drastically reduces the availability of free zinc ions (Sunda and Huntsman, 1998a).

From its concentration profiles, Cd is a recycled element *par excellence*. It exhibits the greatest surface depletion of all metals relative to its deep water concentrations (Saager *et al.*, 1992). For example a 500-fold increase in the Cd_T concentration is observed from the surface to 800 m in depth profiles in the North Pacific (Bruland, 1980). It is also enriched in phytoplankton in surface waters to a greater degree than any other metal, with the exception of iron (Saager, 1994). There is a significant increase in deep water concentrations in moving from the Atlantic (3.5×10^{-10} M) to the Pacific (1.0×10^{-9} M), characteristic of recycled elements. Furthermore, there are strong correlations throughout the world oceans between Cd_T and phosphate (Appendix IIIC) (Boyle *et al.*, 1976; Bruland *et al.*, 1978b; Bruland, 1980; Knauer and Martin, 1981; Bruland and Franks, 1983; Hunter and Ho, 1991).

In many instances, the correlations observed between Cd_T and phosphate are statistically more significant than the correlations between phosphate and nitrate. The relationships are so clear and all-pervading that oceanic cadmium levels (as deduced from analyses of the calcium carbonate shells of foraminifera) have been used as a proxy for oceanic phosphate concentrations when reconstructing the chemistry of ancient oceans from the sedimentary record (Boyle *et al.*, 1976; Bruland *et al.*, 1978b; Hester and Boyle, 1982; Boyle, 1988, 1990). A value of $3.5–4.0 \times 10^{-10}$ M/μM is normally used for the Cd_T:P ratio in these paleo-reconstructions (Boyle, 1988; Broecker and Denton, 1989). Deviations from the linear Cd_T:P trend are observed in surface waters (Boyle *et al.*, 1981), marginal basins (Boyle *et al.*, 1982, 1985; Hunter and

Ho, 1991) continental shelf waters (Boyle *et al.*, 1984) and upwelling regions (Hunter and Ho, 1991).

The Cd_T:P ratio increases with depth, reaching its maximum value at or just below the thermocline. This suggests that Cd is selectively removed from sea water by phytoplankton more effectively than phosphate in the euphotic zone (Kremling and Pohl, 1989) and is released from the particulate matter at greater depths. The deep water ratios observed in the Cd_T:P plots range from 2.0×10^{-10} M/μM in the North Atlantic to 8.7×10^{-10} M/μM in the north-west Indian Ocean. There is a distinct trend in the deep water below 1000 m with the Cd_T:P ratios increasing on following the path of the deep ocean conveyor belt from the Arctic through to the Pacific (Figure 14) (Bruland and Franks, 1983; Saager *et al.*,

Figure 14 The Redfield Cd:P ratio of the deep water plotted as a function of its age. Selected deep water samples (>1000 m) show a consistent increase in the Cd:P ratio as the waters age (higher δ^{14}C values for dissolved inorganic carbon are found in older waters). Reproduced from: de Baar, H. J. W., Saager, P. M., Nolting, R. F. and van der Meer, J. (1994). Cadmium versus phosphate in the world ocean. *Marine Chemistry* **46,** 261–281, with the permission of the copyright holders, Elsevier Science.

1992). This has been attributed to the regeneration of Cd at greater depths than P as the biogenic particles are broken down (Boyle, 1988) so that excess Cd_T aggregates in the deeper water with time (Saager, 1994).

Considerable discussion has arisen over an apparent discontinuity ("kink") in the slope of the Cd_T:P correlation at a phosphate concentration around 1.3 μM, after which the slope of the plot increases significantly. This is an artefact (Elderfield and Rickaby, 2000) that has been attributed to hydrographic features resulting from the conservative mixing of different water masses (Westerlund and Öhman, 1991; Frew and Hunter, 1992). Saager (1994) has analysed a geometric artefact that arises from the superposition of end-members from a continuum of Cd_T:P correlations with gradually increasing slope (de Baar *et al.*, 1994). The main factor controlling the deep water Cd_T:P ratios is the mixing of different water bodies, modulated by the input of cadmium and phosphate from the breakdown of particulate matter and from the sediments. The Cd_T:P correlation shown by all data indicates a very clear trend, with the slopes of the major correlations encompassed within it (Saager, 1994). If the analysis is restricted to high quality data sets (Löscher *et al.*, 1997) from deep waters (>1000 m) driven and mixed by thermohaline circulation (Figure 15) then a tighter correlation is observed with a mean slope of 4.0×10^{-10} M/μM, which is identical with that used in the palaeo-oceanographic analyses (Boyle, 1988). There is an intercept of -2.5×10^{-10} M Cd_T at zero phosphate concentration.

Overall then the Cd_T:P ratios in the deep water of the global ocean are controlled by (1) the existing (or preformed) concentrations of the elements in the water body, (2) the regeneration of the elements when the particulate matter is broken down (either in the water column or in the sediment), and (3) the mixing and transport of the water by the deep ocean circulation. The key difficulty in attributing quantitatively biological mechanisms to the observations is the disturbance of the one-dimensional particle transport view by these advective processes.

6.5.2. *Biological availability of cadmium and impact on productivity*

The stereotypical behaviour of cadmium as a recycled or nutrient element is surprising since it has not hitherto been recognized as a biologically essential element. Culture experiments with diatoms have shown that Cd can act as a substitute for Zn in some enzyme systems (Price and Morel, 1990), including carbonic anhydrase (Lee *et al.*, 1995). This work has been extended to a wider range of phytoplankton species (coastal diatoms, oceanic prymnesiophytes, chlorophytes, dinoflagellates) (Lee and Morel, 1995). Three out of the six species studied that were zinc limited at

Figure 15 Redfield ratios for Cd:P in the world oceans. A plot of selected deep water (>1000 m) data showing a slope of 4.0×10^{-10} M μM^{-1}. ○, North Atlantic (lower group), Antarctic, Indian, Pacific (upper group); ■, South Atlantic; ▲, Tasman Sea. Reproduced from: Löscher, B. M., van der Meer, J., de Baar, H. J. W., Saager, P. M. and de Jong, J. T. M. (1997). The global Cd/phosphate relationship in deep ocean waters and the need for accuracy. *Marine Chemistry*, **59**, 87–93, with the permission of the copyright holders, Elsevier Science.

inorganic zinc concentrations of 3.2×10^{-12} M, were able to grow faster when supplied with 4.6×10^{-12} M of inorganic cadmium. At lower inorganic zinc concentrations (1.6×10^{-13} M), the Cd additions proved toxic to most species. The antagonisms between Cd and other nutrient elements (notably Zn and Mn) also indicate that Cd is readily assimilated by phytoplankton (Sunda and Huntsman, 1996, 1998a).

It has therefore been suggested that Cd uptake could be related to this tendency for Cd to substitute for Zn when zinc itself becomes limiting (Price and Morel, 1990; Morel *et al.*, 1991; Lee *et al.*, 1995). Strong evidence for the substitution of Cd for Zn in carbonic anhydrase has come from studies of natural populations of phytoplankton (mainly large diatoms) in coastal waters off central California (Cullen *et al.*, 1999). Culture experiments with *Thalassiosira weissflogii* have provided unequivocal evidence of the formation of Cd-carbonic anhydrase (Cd-CA) – the only known example of the biological use of Cd. The Cd concentrations in phytoplankton in the field study were shown to be inversely related to the ambient concentrations of carbon dioxide and of Zn in the sea water, providing further complications for the interpretation of the historical record of Cd:P ratios.

It is not known how widespread this zinc substitution phenomenon is. The free concentration of Cd^{2+} in the surface ocean is very low. Cd_T concentrations rarely exceed 10^{-11} M in the surface waters of the Atlantic and the Pacific Oceans (Löscher *et al.*, 1998). Up to 70% of the Cd_T in solution is complexed with organic ligands (Bruland, 1992). The ligand concentration decreases with depth in a manner similar to that observed for Cu-complexing ligands. Of the inorganic Cd remaining some 95% will be held in strong chloro-complexes (Turner *et al.*, 1981). This leaves a free Cd^{2+} concentration of around 3.5×10^{-13} M. Even if the inorganic complexes are also accessible for cadmium uptake by the cell, because of the relatively rapid ligand exchange rates for Cd^{2+} (see Table 5), a high selectivity for Cd over Zn would be required. It is possible that the cadmium is being utilized as rapidly as it is being supplied by the delivery of soluble Cd in atmospheric dust. Whereas some 70% of the input of Cd is by aeolian input, <10% of the Zn is delivered in this way (Duce *et al.*, 1991). Upwelling would also deliver additional supplies of Cd but it would deliver adequate Zn supplies at the same time.

Cd uptake and correlations with phosphate are also noted even where Zn supplies are adequate for the requirements of phytoplankton (Martin *et al.*, 1993). Therefore it seems likely that purposeful uptake by phytoplankton to remedy a zinc deficit is not the only reason for the close correlations observed between Cd and phosphate. Another explanation of the apparently selective uptake of cadmium by phytoplankton may be found in the ability of Cd^{2+} to bind strongly to both polyphosphates (Jensen *et al.*, 1982) and to sulphur-based sites. In phytoplankton, polyphosphates are only encountered under phosphorus-replete conditions, which are rarely found in the natural environment. It is likely that Cd can be assimilated via the mechanisms responsible for the uptake of zinc, but once within the cell its strong binding capabilities make it more difficult to expel. Once within the cell it could also be used to fulfil roles normally taken by zinc if this is

in short supply. If this is the case then it is the relative cell quotas of Cd and Zn rather than their relative concentrations in solution that are important.

6.6. Cobalt

6.6.1. *Distribution of cobalt in the oceans*

Information on the distribution of cobalt in the oceans is sparse (Danielsson, 1980; Knauer *et al.*, 1982; Jickells and Burton, 1988; Martin *et al.*, 1989; Westerlund and Öhman, 1991). The reported surface concentrations range from 4 to 50×10^{-12} M in the North Pacific (Martin and Gordon, 1988; Martin *et al.*, 1989), with typical values of about 25×10^{-12} M in the Atlantic and the Pacific. Cobalt chemistry in the oceans displays some of the characteristics of iron and manganese. It can exist as Co (II) and Co (III) and the higher oxidation state forms highly insoluble oxyhydroxides. While Co (II) is thermodynamically stable in oxygenated sea water, the presence of ferromanganese oxyhydroxide particulates accelerates the deposition of Co (III) (Dillard *et al.*, 1982; Glasby and Thijssen, 1982; Crowther *et al.*, 1983). There is also evidence for the biological oxidation of Co (II) to Co (III) (Lee and Fisher, 1993a). The vertical profiles therefore show evidence of scavenging with a slight (30%) depletion in the deep water relative to the surface values. Cobalt, like iron, has a significant hydrothermal source (equivalent to the input from the rivers), but it will also be readily oxidized to Co (III) at the surface of iron and manganese oxyhydroxides formed in the rising plumes.

Cobalt is not strongly complexed by inorganic ligands (Turner *et al.*, 1981), with approximately 60% of the cobalt being present as Co^{2+} in sea water at 25°C (pH 8.1). However, it does form strong and specific complexes with organic ligands – vitamin B_{12} (cobalamin) being a particularly selective example. Organic complexes (Donat and Bruland, 1988; Zhang *et al.*, 1990) can reduce the concentration of free Co^{2+} to values approaching 10^{-15} M. The formation of such complexes, however appears to enhance the ability of organisms to assimilate cobalt, as was the case with iron. There have been no reports of correlations between cobalt and other nutrient elements.

6.6.2. *Biological availability of cobalt and impact on productivity*

As the active metal centre of vitamin B_{12}, cobalt is an essential growth factor for phytoplankton (diatoms, chrysophytes, dinoflagellates) (Swift, 1980) and its availability has been suggested to play a part in the seasonal succession of phytoplankton species (Swift, 1981). The ability of algae to engineer the vitamin B_{12} molecule to make it less readily available to other species (Pitner and Altmeyer, 1979) suggests another variation on the

"chemical warfare" concept that was discussed with respect to the sequestration of iron via species-specific siderophores.

There is some evidence that Co can substitute for Zn when this is in short supply. However, the low ambient concentrations of Co_T suggest that this will be a device of limited value in natural systems. Nevertheless, it could have implications for the structuring of phytoplankton ecosystems in some circumstances (Price and Morel, 1990; Sunda and Huntsman, 1995a). Culture experiments show that a high Co^{2+}:Zn^{2+} ratio favours the growth of *Emiliania huxleyi* over diatoms when Zn_T concentrations are low. The highest values of this ratio (0.4) occur where coccolithophorids dominate. Diatoms are favoured by the high Zn^{2+} concentrations and low Co^{2+}:Zn^{2+} ratios that are found in nutrient-rich upwelling waters. Since these two categories of phytoplankton have quite different biogeochemical impacts the factors controlling their relative preponderance are of considerable significance. Active scavenging of Co above the thermocline has been observed (Martin and Gordon, 1988; Martin *et al.*, 1989) when Zn_T levels fall below 3×10^{-10} M ($\equiv 3.2 \times 10^{-12}$M free Zn^{2+}). At these levels the culture experiments show a high induction rate for Co uptake.

6.7. Nickel

6.7.1. *Distribution in the oceans*

Nickel is not affected by redox processes (Jacobs *et al.*, 1987; Haraldsson and Westerlund, 1988) and it shows a typical nutrient profile with surface depletion relative to deep water concentrations. However, the surface concentrations always remain high ($>1 \times 10^{-9}$ M) and the deep to surface concentration ratios are never >10. Profiles measured in the Pacific Ocean (Sclater *et al.*, 1976; Bruland, 1980; Boyle *et al.*, 1981), the Indian Ocean (Saager *et al.*, 1992) and the Atlantic Ocean (Bruland and Franks, 1983; Yeats and Campbell, 1983; Danielsson *et al.*, 1985) show an increase in the deep water concentrations on moving from the Atlantic to the Pacific (Figure 16). The profiles do not show very strong correlations with P or Si (Appendix IIID) (Danielsson, 1980; Saager *et al.*, 1992). Mixed correlations with both Si and P simultaneously (Nolting and de Baar, 1990) give the statistically most significant fits, but the relationships are not very convincing from the biogeochemical point of view (Saager, 1994).

Nickel is only weakly bound by inorganic complexes (50% present as Ni^{2+}) (Turner *et al.*, 1981). There is some evidence of organic complexation (van den Berg and Nimmo, 1987; Nimmo *et al.*, 1989) by relatively small concentrations of strong binding ligands that can complex up to half of the total nickel.

Figure 16 Vertical concentration profiles for nickel in the world oceans. ■, North Atlantic Ocean; ○, east equatorial Atlantic Ocean; ▲, west equatorial Indian Ocean; □, Antarctic Ocean; ●, north-west Indian Ocean; △, North Pacific Ocean. (Reproduced with the permission of the copyright holders from: Saager, P. M. (1994). "On the relationships between Dissolved Trace Metals and Nutrients in Seawater". CIP-Gegevens Koninklije Bibliotheek, The Hague.)

6.7.2. *Biological availability and impact on productivity*

Nickel is an essential co-factor in urease. Urea can be an important source of nitrogen after the first flush of nitrate assimilation has died down and the grazing animals have begun to release their waste products. Up to half of the nitrogen for photosynthesis may be acquired from urea under these conditions (McArthy *et al.*, 1977; Harrison *et al.*, 1985). Phytoplankton cultures growing on urea as a nitrogen source can be limited by low free nickel concentrations (Price and Morel, 1991). There have been no reports of such limitation in natural systems, probably because of the high background levels of nickel.

7. DISCUSSION

The study of phytoplankton interactions with trace metals in the oceans confirms the powerful biological influences at work. The observations do however raise some questions about the degree to which the system has become optimized to meet the requirements of phytoplankton.

7.1. Fractionation of the elements in the oceans

The vertical concentration profiles of most of the elements dissolved in sea water (the recycled and scavenged elements) are dominated by the combined effect of uptake by phytoplankton in the surface layers, vertical transport into deep water and release by the microbial breakdown of organic particles. Although the essential trace metals have been the focus of this review, many other elements are entrained by the biologically driven particle cycles within the oceans (Appendix I).

7.1.1. *Indices of fractionation*

The most straightforward index of the fractionating power of this biological particle pump is the ratio of the deep to surface concentrations in the Atlantic and Pacific Oceans at either end of the oceanic conveyor belt. A plot of these ratios (Figure 17, see Appendix I) indicates a considerable degree of fractionation for more than 30 elements. In order to present the plot for most elements on a reasonable scale (Figure 17B), five elements (Se, Ge, Si, P, Cd) had to be omitted from those shown in Figure 17A because of the large fractionation ratios (deep:surface concentrations) that they exhibit (ranging from 12 to 150, Appendix I). The correlation between the fractionation ratios in the two oceans is not very significant. Most of the points lie above the 1:1 trend line, indicating an enhanced degree of fractionation in the Pacific. It is clear that biological factors have a dominating influence on the distribution of these elements.

Broecker and Peng (1982) have used a two box model of the world ocean to estimate the average removal flux of elements from the surface layers in biologically generated particles. The total flux of elements into the "euphotic zone box" is assumed to equal the total flux out. The flux of elements out of the "deep ocean box" into the sediment is assumed to match the flux of elements into the surface box from the rivers. A comparison of the fraction (g) of the element entering the surface layers that is removed by particles in the Atlantic and in the Pacific will give an indication of the impact of the particle pump. At steady state (and ignoring

Figure 17 The fractionation of the elements in the global ocean by the biological particle pump. (A) A plot of the deep:surface concentration ratios for all elements (Appendix I): $y = 2.4x - 0.44$ ($r^2 = 0.49$). (B) An enlarged plot with the elements identified in (A) omitted. For clarity only biologically essential elements have been identified: $y = 2.17x - 0.18$ ($r^2 = 0.43$). The solid line represents the linear regression and the broken line the 1:1 correlation.

atmospheric and hydrothermal contributions), Broecker defines the mixing flux of water between the surface and deep boxes as being about 30 times the river flux. Using a value of 3.7×10^{16} kg yr^{-1} for the mean annual river flow and the data for ocean composition (Appendix I) and river inputs (Appendix II), g-values have been calculated for the Atlantic and the Pacific (Figure 18). Again this plot confirms the major impact that biological cycling has by processing a large fraction of the minor and trace elements through the particle pump. As well as the essential elements, many other elements (e.g. the rare earth elements) are entrained in this process. Vigorous recycling is therefore not necessarily an indicator of biological significance.

A plot of the particle flux versus the flux of elements brought to the surface in upwelling waters has also been used to illustrate the dominant effect of biological cycling on the fractionation of the elements (Whitfield, 1981). It would be worthwhile to repeat this exercise using the large volume of data that has been produced on the fractionation of the elements through the planktonic ecosystem. This would include trace metal removal:

Figure 18 The fraction (g) of the elements entering the surface waters of the Atlantic and the Pacific that is removed by biologically produced particles (Broecker and Peng, 1982). Calculated from a two-box model of the world ocean, which is assumed to be running at a steady state over a period of a million years or more. Only the essential elements (●) are identified for clarity. The broken line is the 1:1 relationship and the solid line the linear regression: $y = 0.775x + 0.263$ ($r^2 = 0.63$).

1. through the foodweb – e.g. via copepods (Reinfelder and Fisher, 1991), zooplankton grazing (Elser and Hasett, 1994; Hutchins and Bruland, 1994, 1995), and trophic transfer (Fisher and Reinfelder, 1995);
2. through interactions with detrital particulate matter – faecal pellets (Fowler, 1977; Fisher *et al.*, 1991), biogenic particulates (Collier and Edmond, 1983, 1984; Lee and Fisher, 1994), phytoplankton debris (Lee and Fisher, 1992a; Fisher and Wente, 1993; Lee and Fisher, 1993b) and zooplankton debris (Lee and Fisher, 1992b; Reinfelder *et al.*, 1993).

Sediment trap studies have also provided a wealth of data on the trace metal composition of particulates sinking through deep water (see for example the compilation of Saager, 1994).

7.1.2. *The cycling ratio*

Volk (1997) has considered the possibility of delineating the influence of the biosphere by relating the recycling of the elements through ecosystems to the external flow of elements through their environment. A cycling ratio (R_Y) can be defined for the element Y where:

R_Y = (flux circulating within the ecosystem)/(flux circulating through the environment)

Volk suggests that the intensity of recycling should provide a measure of the extent to which the biological system employs the energy it can derive from external sources to optimize its utilization of the geochemical cycles. If correct, the concept could be extremely valuable in defining the effective limits of the cycling of the elements by biological systems. The massive capabilities of phytoplankton to redistribute the elements in the oceans would seem to provide an excellent context for testing the value of this concept for rationalizing the observations.

If we take the world ocean as the environment of interest, the flux circulating into the environment can be calculated from the known inputs from rivers, from hydrothermal vents and from the atmosphere (Appendix II). The flux circulating within the ecosystem can be approximated by the flux of the elements required to maintain global marine primary production. Element to carbon ratios are best obtained directly from analyses of phytoplankton (Table 15). The latest estimates of global marine primary production (Field *et al.*, 1998) can then be combined with the relevant element to carbon ratios (Table 14) to give calculated biological fluxes.

Table 15 Phytoplankton composition and threshold values for growth.

	Phytoplankton composition[a]						Threshold values[b]	
	Martin *et al.*, 1976 $n = 9$		Martin and Knauer, 1973 $n = 4$		Collier and Edmond, 1983 $n = 2$		Morel and Hudson, 1985	
Element	M_T[c]	M:P[d]	M_T	M:P	M_T	M:P	nutrient	toxic
P	260		250		280		300	
Ca	480	1846	160	640	1400	5000		
Si	1600	6153	1000	4000	470	1678		
Al	2	7.69	1.2	4.8	0.83	2.96		
Fe	1.3	5.00	1.3	5.2	1.3	4.64	3	
Zn	0.47	1.81	0.21	0.84	0.83	2.96	0.2	15–30
Mn	0.1	0.38	0.097	0.388	0.096	0.34	0.4–2	
Cu	0.1	0.38	0.05	0.2	0.15	0.54		5–50
Ni	0.09	0.35	0.05	0.2	0.24	0.86		2
Cd	0.12	0.46	0.017	0.068	0.15	0.54		0.2

[a]Phytoplankton composition data taken from the compilation by Bruland *et al.*, 1991.
[b]μmol g^{-1} of dry weight.
[c]M_T = total concentration in cell (μmol g^{-1} of dry weight).
[d]Ratios in mmol mol^{-1} of P.

The calculated values of the cycling ratio are surprising at first sight (Table 16). Although the essential macronutrients C, N, P and Si do show large cycling ratios (>10), the essential trace metals Cu, Zn, Mn and Fe show low values (<2). Furthermore, elements for which there is only a small requirement (Cd, Ni) have cycling ratios as large as the macronutrients. In fact the cycling ratio for Cd is the largest of all, being more than twice that of phosphorus!

It would seem that the cycling ratio is only telling part of the story. There is in fact a clear and simple relationship between R_Y and T_Y (the mean oceanic residence time of the element Y, see Figure 19); a log-log plot gives a significant correlation between the two. Primary production, directly or indirectly, controls the packaging of the elements into particles and their rapid transport into the deep ocean. The settling of the particles onto the sediment is the main route for their removal from the oceans. Where the geochemistry works in its favour, the biological system can recycle the elements very effectively and reduce the proportion of the elements that are deposited into the sediment on each stirring cycle of the oceans. Where the geochemistry is removing the elements effectively by scavenging, the organisms instead have to resort to efficient and parsimonious use of these elements.

Table 16 Calculation of the cycling ratio (R_Y) for phytoplankton cells.

Element	M:C ratio[a] (μmol mol^{-1} C)	Biological flux[b] (10^{11} mol yr^{-1})	Total input flux[c] (10^{11} mol yr^{-1})	Cycling ratio (R_Y)	Log T_y[d]
Cd	3.5	0.14	3×10^{-4}	494	4.6
P	9400	385	1.8	214	4.3
C	10^6	4083	750	54	4.6
N	6.3×10^4	2710	82.3	33	3.8
Ni	3.5	0.15	5×10^{-3}	30	4.4
Si	4.7×10^4	1920	130	15	4.1
Cu	3.4	0.14	0.06	2.2	2.9
Al	59	2.4	1.3	1.9	1.8
Zn	16	0.65	0.3	1.9	2.4
Fe	47	1.9	1.1	1.8	1.7
Mn	3.6	0.15	0.1	1.4	2.2

[a]Taken from Tables 13 and 15 (weighted means = $106*\{\Sigma n*\text{M:P}/\Sigma n\}$) and based upon measurements on phytoplankton samples.
[b]Based on an annual primary production (Field *et al.*, 1998) of 49×10^{15} g C yr^{-1}.
[c]River + vents + aeolian (Appendix IIA). The value for C is based on river input values from Butcher *et al.* (1992). The value for N includes river input (Butcher *et al.*, 1992) and N fixation (Tyrell, 1999).
[d]Values recalculated from mean oceanic concentrations (see Appendix I) and total input fluxes (Appendix II).

The cycling ratio is therefore not related primarily to the requirement that phytoplankton have for the element in question. This is masked by two effects. The first factor is the inability of phytoplankton to exercise absolute discrimination in their quest for essential nutrients. Elements entering as *faux amis* will be recycled as effectively as essential nutrients. So long as they do little harm to the viability of the cells, they will not exert any selective pressure against their uptake. Cadmium is an excellent case in point. The second factor is that phytoplankton are capable of actively regulating their internal economy for the trace metals. Some elements are present in such low concentrations because of geochemical factors that the physiology of the cells is stretched to the limit. For most essential trace metals the cells are at, or close to, their nutrient limitation levels (Table 15). Under such circumstances the internal cell quota (Q_Y) is minimized and the Y:C ratio will be disproportionately low. To assess the extent of biological manipulation of the environment we must look more closely at the impact on the availability of the essential elements.

Figure 19 The correlation between the recycling ratio (R) and the mean oceanic residence time (T_y) for essential elements. The solid line represents the linear regression $y = 0.701x - 1.226$ ($r^2 = 0.80$). Data are taken from Table 16 where detailed notes are given.

7.2. Feedbacks in the system

7.2.1. *Classification*

The influence of biological processes goes beyond the simple mass transport of the elements in the ocean. The productivity of phytoplankton depends *inter alia* on the availability of the essential macro- and micronutrients. The evidence suggests that phytoplankton can regulate the quality of their culture medium by influencing the availability of the essential trace metals. There has been an intimate interlocking of the biological requirements for the supply of nutrients to match the internal demands of phytoplankton (the internal economy) and the distribution and relative concentrations of the elements in the marine environment (the external economy). By striving to maintain adequate supplies of essential nutrients at the cell surface the organisms directly affect the near-field chemistry of the elements. Because of the mobile and interconnected nature of the oceans, these local perturbations eventually have an impact on the regional

and then the basin-wide scale, thereby defining the far-field chemistry of the marine system.

Lenton (1998a) has identified three intrinsic properties of living organisms that together characterize their potential for interacting with their environment:

1. All organisms alter their environment by taking in free energy and excreting high-entropy waste products in order to maintain a low internal entropy.
2. Organisms grow and multiply, potentially exponentially, providing an intrinsic positive feedback to life (the more life there is, the more life it can beget).
3. For each environmental variable, there is a level or range at which growth of a particular organism is maximum.

These characteristics interact with the fundamental extrinsic property that:

> . . . once a planet contains different types of life (phenotypes) with faithfully replicated, heritable variation (different genotypes) growing and competing for resources, natural selection determines that the types of life that leave the most descendants come to dominate their environment. (Lenton, 1998a)

The consequence is a hierarchy of feedbacks that arise from the interaction of living organisms with their environment.

1. Abiotic (geochemical) feedbacks – present on a lifeless planet.
2. Growth feedbacks – organisms alter their environment in a manner that affects their growth, without altering the forces of selection on the responsible trait.
3. Selective feedbacks – traits that alter the forces of selection on themselves. These feedbacks involve selection. Selective feedback occurs whenever the spread of a trait critically alters the environmental variable that determines the benefit of that trait.

The conclusions of this review will be considered briefly in the context of growth feedbacks and selective feedbacks.

7.2.2. *The internal economy of the cell and the near-field chemistry*

Culture studies show that phytoplankton are usually able to regulate their cell quotas of essential trace metals (see for example Figures 11 and 13) by negative feedback control of the concentration of surface ligands (L1, Figure 4). The metal:C ratios observed in the culture experiments are

comparable to those determined in phytoplankton samples from the surface ocean, provided that the ambient concentration of the appropriate free metal ion is taken into account (Figure 13). These ratios are also similar to those estimated from water column metal:P ratios (see the data for Cu in Table 14, and also the discussion on Cd, and Appendix IIIC). Calculations on the interactions between trace metals indicate a significant degree of consistency between culture experiments and the simultaneous influence of several trace metals on phytoplankton growth in the natural system.

This regulatory growth feedback, together with the interactions between the metal cycles that are known to exist within the cells (see Figure 3), suggest a regulated internal economy that is seeking to optimize its interactions with a suite of potentially limiting elements (Morel and Morel-Laurens, 1983; Morel and Hudson, 1985; Bruland *et al.*, 1991; Morel *et al.*, 1991). In the open ocean, cells are working close to the diffusion limit for the uptake of trace metals (see Table 15) and therefore have had to reduce their intracellular requirements to a minimum and to seek substitutions wherever possible. The correlation illustrated in Figure 5 indicates the close coupling that exists between the concentration of available metal in the surface layers of the ocean and the properties of the metal uptake mechanism. The marked differences in the capabilities of coastal and oceanic eukaryotes (Tables 10 and 15) and between prokaryotes and eukaryotes in this regard suggest that these local growth feedbacks are also acting as selective feedbacks on the oceanic scale.

A further example of the internal economy of the cell imprinting itself on the bulk chemistry of sea water is provided by the release of organic complexing agents into solution. The effect of organic complexation on phytoplankton appears to be beneficial for iron (by enabling a large pool of dissolved iron to be maintained) and for copper (by maintaining the concentration of Cu^{2+} below the toxic limits). The ligands binding copper are readily broken down by microbial action below the euphotic zone, whereas those binding iron appear to be quite stable to microbiological attack. However, photochemical processes involving the ligands in the surface waters do enable a supply of Fe (II) and Fe (III) to be maintained (Figure 8). Beneficial effects are also noted for manganese, although here the photoreduction of Mn (IV) to Mn (II) takes place at particle surfaces in the presence of dissolved organic matter.

The effect of organic complexation on zinc appears to be detrimental. It reduces the accessible level of Zn^{2+} to the point where the uptake requirements for the cells can only just be met by taking up the ions at the maximum rate allowed by physical diffusion. Any further adaptation to low zinc levels can only be achieved by reducing the internal requirements for zinc. Is this an inevitable if accidental consequence of the release of

organic compounds? It is interesting to note that both copper and zinc can act either as essential or toxic elements within the concentration ranges that are normally encountered in natural waters. There may be some carefully balanced damage limitation exercise at work here. The benefits provided by the regulation of the availability of Fe and Cu outweigh the disadvantages associated with difficulty in obtaining sufficient zinc.

The example of cadmium is fascinating. It is recycled more effectively than any nutrient element and yet it does not appear to be essential to life. Indeed it is a widely recognized toxic element. Although there are some beneficial substitutions noted for zinc, it would appear that the avid collection of cadmium by phytoplankton cells is an accidental consequence of their essential metabolic processes. Cadmium binds very effectively to pyrophosphates and to sulphur-based ligands within the cells. It is also very labile to ligand exchange so that the cells will readily take it up. It is not as strongly bound by organic complexes in solution as iron, copper or zinc. This state of affairs can be tolerated so long as the ambient cadmium concentrations remain low. It does however make it difficult to judge the significance of biological feedbacks by gauging the intensity of biological recycling.

7.2.3. *The influence of community structure*

It is apparent that while phosphorus is the ultimate limiting nutrient for primary productivity as a whole, nitrogen is a proximate limiting nutrient (Tyrell, 1999). Although ammonium ions or urea remain the preferred forms of nitrogen for uptake by phytoplankton, they are able to utilize nitrate or nitrite at an additional metabolic cost. In the absence of the industrial fixation of atmospheric nitrogen (Galloway *et al.*, 1995), the inexorable progress of the denitrifying organisms releasing N_2 and N_2O in oxygen-poor environments is counterbalanced to some degree by the strenuous efforts of the nitrogen-fixing cyanobacteria in other areas and by the production of nitrate ions by electrical storms. The feedback systems linking these processes to the utilization of phosphate and the release of oxygen imprint the internal economies of the primary producers onto the far-field chemistry of the atmosphere and of the oceans. These processes are modulated by trace metals.

Both nitrogen fixation and denitrification depend upon the activity of metal-containing enzymes. It is also likely that the availability of trace metals is a significant factor in regulating primary productivity. Iron is a key factor here, particularly for nitrogen fixation and photosynthesis by prokaryotes in oligotrophic and HNLC regions. The Fe and Si cycles are

also intertwined in a way that can reduce the potential for iron additions to induce diatom blooms.

Williams (1996) has suggested that the way in which organisms imprint their requirements for the essential elements will be best understood by investigating the internal links between these elements within the cell via the functioning of enzymes.

> (The) interaction of the global cycles of nitrogen and carbon takes place at the biological level. The internal metabolic processes that lead from the inorganic nutrients carbon dioxide and ammonia to biomass assimilation meet in the enzymic steps in which nitrogen is incorporated into glutamine. The rate of this incorporation is controlled by the activity of a key enzyme, glutamine synthetase. The formation of ammonia from nitrate . . . is catalysed by nitrate reductase. We have seen how the activity of this enzyme is regulated, and coordinated to that of glutamine synthetase, by the supply of fixed forms of nitrogen. Similarly, the activities of the enzymes responsible for the reduction of nitrogen to ammonia, are finely tuned to the needs of the nitrogen-fixing microorganisms.
>
> It thus appears that the interaction at the biological level of the vast global biogeochemical cycles of carbon and nitrogen may be a reflection of events at the molecular level. One might assert that the regulation of the global biogeochemical cycles is the molecular control of intermediary metabolism writ large. From this perspective, global metabolism would be seen to be just as much a result of the properties of proteins synthesized under the control of genes, as is cellular metabolism. (Reproduced from: "The Molecular Biology of Gaia", by G. R. Williams (p. 179). Columbia University Press, Reprinted by permission of the publisher.)

The value of this perspective is considerably reinforced when the cycles of the essential trace metals are taken into account.

The release of DMS and the export of particulate carbon (as organic carbon and as $CaCO_3$) from the surface layers (and hence the net drawdown of CO_2 from the atmosphere) are intimately linked with ecosystem structure. The physical forcing functions controlling the stability of the surface mixed layer play a major part in defining the ecosystem structure (Figure 20) by influencing the characteristic size and lifestyles of the primary producers (Legendre and Le Fèvre, 1989). At one extreme, intermittent physical mixing enhances nutrient supplies and encourages the growth of large, bloom-forming diatoms when the water column restabilizes. These are grazed in turn by relatively large zooplankton whose faecal pellets will aggregate and fall rapidly from the surface layers into deeper water. At the other extreme, strongly stratified water columns encourage the growth of small cyanobacteria and prochlorophytes, which are grazed by small protozoa. A parsimonious ecosystem results with very little transport of faecal material into deeper water. Trace metals, because

of their differential impact upon different species, can also influence ecosystem structure (see for example Table 9).

There are three key ratios (Figure 20) that can be used to characterize the ecosystems with respect to their impact on these feedback processes:

1. The export ratio that defines the proportion of carbon fixed in particulate organic matter that is exported from the surface layers into the deep ocean (Eppley, 1989; Baines *et al.*, 1994).
2. The rain ratio that defines the ratio of organic carbon to carbon as calcium carbonate transported in particulate matter from the euphotic zone (Broecker and Peng, 1982; Tsunogai and Noriki, 1991; Denman and Peña, 2000).
3. The Redfield ratio that defines the stoichiometric relationships between the nutrient elements and carbon in the microbial ecosystems and the resulting detrital particulate matter and influences the composition observed in the deep water (Elser and Urabe, 1999).

The shifts in ecosystem structure brought about by climate change (Karl, 1999) might themselves introduce additional feedbacks here. For example, global warming would be expected to result in an increase in the input of

Figure 20 Interactions between external forcing functions, including climatic change (open arrows) and the structure of oceanic ecosystems (closed arrows).

heat to the surface layers of the ocean and hence an extension of the area covered by oligotrophic regions (Lovelock and Kump, 1994). This in turn will reduce the export of organic carbon to the deep ocean through the associated shift in ecosystem structure. The net result would be a reduction in the draw-down of CO_2 into the oceans (Roemmich and McGowan, 1995), and hence an intensification of greenhouse warming. The potential for shifts in the efficiency of metal-containing enzyme systems has also been suggested (Beardall *et al.*, 1998). On the other hand increased heat input to the surface oceans will result in increased water evaporation, resulting in an increasing incidence of storms and therefore enhanced mixing. The overall balance of such effects is unknown, although feedbacks through the community structure will be felt at both extremes. The complexity of such effects is well illustrated by recent studies of the effect of vertical mixing on the production of DMS in surface waters through the impacts on community structure (Kiene, 1999; Simó and Pedrós-Alló, 1999).

7.3. The resilience of ocean ecosystems

Overall, the growing mass of evidence points towards a major global role for unicellular life in generating feedbacks which help to regulate the environment upon which it depends. According to Volk (1997) biological feedbacks arise from "no-cost by-products of local self-centred organismic evolution". He further suggests that:

> What organisms do to help themselves survive may alter the planet in enormous ways that are not at all the reasons for these survival skills being favoured by evolution.

The impact of the release of organic matter from phytoplankton and non-photosynthetic bacteria provides some interesting examples of the adaptation process at work. The release of such material is inevitable as the cells die and are lysed or are eaten. The material exuded in this way would be primarily cell contents produced to serve the internal economy of the cell. This will include compounds such as hydrolytic enzymes and metal complexing agents used to regulate the concentration and flow of metals within the cells. These will interact with the inorganic trace elements in solution that are the prime source of essential metals for the living cells. The overall impact of complexation varies from metal to metal. This process is simply a consequence of the life cycles of the organisms. If it has overall beneficial effects then the ecosystem can continue to thrive. If the effects are detrimental then the ecosystem will be constrained or it will

be replaced by a combination of organisms better able to make use of the resources provided in this way.

These feedbacks can provide components of a control system. However, since they are assembled from the secondary consequences of responses to immediate selective pressures upon individual organisms, such feedbacks will not necessarily provide a regulatory system for the greater good of the majority of species present – although there may be an inexorable trend in this direction. Feedbacks that produce a negative impact on large sectors of the ecosystem might still persist if they convey a large benefit to their perpetrators – especially if other organisms can evolve quickly enough to take advantage of the new conditions. The transition to an atmosphere containing high levels of oxygen is an example of selective feedback that provides a clear example of the risks involved and the benefits that can ensue.

The network of such interactions will gradually strengthen as the selection process takes effect. If an interaction is strongly beneficial it will be reinforced, if it is strongly detrimental it will be eradicated. If, however, it is slight or neutral in its impact it may persist while other factors dominate the selection process. In this way the system can evolve by notching up its successes, eliminating its failures and biding its time on the inconclusive experiments. If a number of these neutral or slight interactions link up to provide a stronger feedback then the system might once more respond decisively. The kind of network that results is similar to the "small-world" networks that provide an intermediate between regular and random networks, with a high degree of connectivity (Watts, 1999). The major characteristic of such networks is the interlacing of clusters of tightly coupled local networks (the near-field interactions) with occasional far-reaching links (the far-field interactions) that provide direct communications between remote components of the overall network. These networks do not have an organizational centre and yet they can generate "global" interactions throughout the network. It would be worthwhile analysing the feedbacks that have been shown to exist between organisms and their environment to see whether the resilience of the resulting network supports the global picture implied by the Gaia hypothesis.

The microbiological community is continuously pushing at the boundaries by optimizing the use of the available energy sources. This leads to the observation of nutrient co-limitation on the growth of phytoplankton rather than the over-riding predominance of a single factor. It also implies that the ability of phytoplankton to feedback on the environment has its limitations. At this level, the feedbacks focus on the interactions between solution chemistry, primary production and particle flux.

Figure 21 Interconnecting circles of influence relating primary production to the trace metal chemistry of the oceans. White sectors represent interfaces where there is a two-way interaction between the factors identified. The central area where all circles intersect represents the situation in the real oceans!

The great phyletic diversity of phytoplankton, the major differences between prokaryotic and eukaryotic cells (Table 1) and the fluid nature of the marine environment all provide great flexibility for marine ecosystems to respond to and modulate environmental changes. The sheer diversity of the plankton itself presents a paradox (Hutchinson, 1961; Siegel, 1998) – how can so many different kinds of phytoplankton coexist on only a few potentially limiting resources in a relatively uniform and fluid environment? Competition theory suggests that there should be only a few competitive winners. Fuhrman (1999) has suggested that viral activity could favour the continuing existence of many rare species since a critical population density is required for successful viral infection. Differential sensitivities to a range of trace metal availabilities may be another factor – especially when linked to the potential chemical warfare effect. The degree of manipulation of the chemical environment identified by this review also suggests that there is a complex and competitive chemical environment intermeshing with the constraints provided by physical and biological pressures. Organisms can compete in terms of

their differential abilities to manipulate their cellular burdens of a suite of essential trace metals as well as their abilities to enhance the availability of essential elements in their immediate vicinity, while at the same time denying their competitors access to these resources. This indicates that there is a wide range of potentially limiting constraints encouraging a diversity of species.

The presence of the mesh of feedbacks provided by the microbiological communities within the oceans has conferred a stability and a resilience on marine life. It is this underpinning by a multitude of active, interconnected and rapidly reproducing organisms that enables the ecosystem to bounce back rapidly from major and prolonged catastrophes resulting from meteorite impacts and extensive volcanism (Knoll, 1989; Falkowski and Raven, 1997). The metazoans come and go, reaping the benefits of the industrious work of the resilient micro-organisms (Ward and Brownlee, 2000), which regulate the basic fitness of the fluid environment for life (Lovelock, 1988).

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APPENDIX I

Elements in the Oceans

		Concentrations (M)					
		Atlantic		Pacific			
Element	Ox	surface	deep	surface	deep	T_y (yr)	Reference*
Accumulated elements							
B	III	4.2×10^{-4}				1.0×10^{7}	Bruland, 1983
Br	-I	8.4×10^{-4}				1.0×10^{8}	Whitfield, 1981
Cl	-I	5.3×10^{-1}				4.0×10^{8}	Whitfield, 1981
Cs	I	2.3×10^{-9}				6.0×10^{5}	Bruland, 1983
F	-I	6.8×10^{-5}				4.0×10^{5}	Whitfield, 1981
K	I	1.0×10^{-2}				5.0×10^{6}	Bruland, 1983
Li	I	2.6×10^{-6}				2.0×10^{6}	Stoffyn-Egli and Mackenzie, 1984
Mg	II	5.3×10^{-2}				1.0×10^{7}	Bruland, 1983
Mo	VI	1.07×10^{-7}				6.0×10^{5}	Collier, 1985
Na	I	4.7×10^{-1}				1.0×10^{8}	Bruland, 1983
Rb	I	1.4×10^{-6}				8.0×10^{5}	Bruland, 1983
S	VI	2.8×10^{-2}				8.0×10^{6}	Whitfield, 1981
Tl	I	6.5×10^{-11}	6.0×10^{-11}	7.0×10^{-11}	8.0×10^{-11}	1.0×10^{4}	Flegal and Patterson, 1985; Donat and Bruland, 1995
U	VI	1.35×10^{-8}				3.0×10^{5}	Anderson, 1982
Recycled elements							
Ag	I			1.0×10^{-12}	2.3×10^{-11}	5.0×10^{3}	Martin *et al.*, 1983
As	V	2.0×10^{-8}	2.1×10^{-8}	2.0×10^{-8}	2.4×10^{-8}	9.0×10^{4}	Andreae, 1979 Burton *et al.*, 1983
Au	III	1.0×10^{-13}		1.0×10^{-13}			Donat and Bruland, 1995
Ba	II	3.5×10^{-8}	7.0×10^{-8}	3.5×10^{-8}	1.5×10^{-7}	1.0×10^{4}	Collier and Edmond, 1984
Be	II	1.0×10^{-11}	2.0×10^{-11}	4.0×10^{-12}	2.5×10^{-11}	4.0×10^{3}	Measures and Edmond, 1982
C	IV	2.0×10^{-3}	2.2×10^{-3}	2.0×10^{-3}	2.4×10^{-3}	8.0×10^{5}	Collier and Edmond, 1984
Ca	II	1.0×10^{-2}	1.1×10^{-2}	1.0×10^{-2}	1.13×10^{-2}	1.0×10^{6}	Bruland, 1983
Cd	II	1.0×10^{-11}	3.5×10^{-10}	1.0×10^{-11}	1.0×10^{-9}	3.0×10^{4}	Collier and Edmond, 1984
Cr	VI	3.5×10^{-9}	4.5×10^{-9}	3.0×10^{-9}	5.0×10^{-9}	1.0×10^{4}	Campbell and Yeats, 1981; Murray *et al.*, 1983
Cu	II?	1.3×10^{-9}	2.0×10^{-9}	1.3×10^{-9}	4.5×10^{-9}	3.0×10^{3}	Collier and Edmond, 1984
Dy	III	5.0×10^{-12}	6.1×10^{-12}			300	Elderfield and Greaves, 1982
Er	III	3.6×10^{-12}	5.3×10^{-12}			400	Elderfield and Greaves, 1982
Eu	III	6.0×10^{-13}	1.0×10^{-12}	7.0×10^{-13}	1.8×10^{-12}	500	Elderfield and Greaves, 1982; de Baar *et al.*, 1985
Fe	III	2.0×10^{-9}	7.0×10^{-9}	2.0×10^{-10}	2.0×10^{-9}	98	Symes and Kester, 1985; Collier and Edmond, 1984

APPENDIX I (contd)

		Concentrations (M)					
		Atlantic		Pacific			
Element	Ox	surface	deep	surface	deep	T_y (yr)	Reference*
Fe		1.3×10^{-9}	8.0×10^{-10}	2.6×10^{-10}	7.5×10^{-10}		Donat and Bruland, 1995
Ga	III	2.7×10^{-11}		1.2×10^{-11}	3.0×10^{-11}		Donat and Bruland, 1995
Gd	III	3.4×10^{-12}	6.1×10^{-12}	4.0×10^{-12}	1.0×10^{-11}	300	Elderfield and Greaves, 1982 de Baar *et al.*, 1985
Ge	IV	1.0×10^{-12}	2.0×10^{-11}	5.0×10^{-12}	1.0×10^{-10}	2.0×10^{4}	Froelich *et al.*, 1985
Hf	III	4.0×10^{-13}	8.0×10^{-13}	3.0×10^{-13}	1.5×10^{-13}		Donat and Bruland, 1995
Ho	III	1.5×10^{-12}	1.8×10^{-12}	1.0×10^{-12}	3.6×10^{-12}		de Baar *et al.*, 1985; de Baar *et al.*, 1983
I	V	2.0×10^{-7}	4.5×10^{-7}	3.5×10^{-7}	4.7×10^{-7}	3.0×10^{5}	Elderfield and Truesdale, 1980; Wong and Brewer, 1974
La	III	1.3×10^{-11}	2.8×10^{-11}	1.9×10^{-11}	5.1×10^{-11}	200	Elderfield and Greaves, 1982; de Baar *et al.*, 1983
Lu	III	8.0×10^{-13}	1.2×10^{-12}	3.5×10^{-13}	2.4×10^{-12}	4.0×10^{3}	de Baar *et al.*, 1985; de Baar *et al.*, 1983
N	V	5.0×10^{-9}	2.0×10^{-5}	5.0×10^{-9}	4.0×10^{-5}	6.0×10^{3}	Takahashi *et al.*, 1981
Nd	III	1.3×10^{-11}	2.3×10^{-11}	1.3×10^{-11}	3.4×10^{-11}	500	Elderfield and Greaves, 1982 de Baar *et al.*, 1983
Ni	II	2.0×10^{-9}	7.0×10^{-9}	2.0×10^{-9}	1.0×10^{-8}	8.0×10^{4}	Collier and Edmond, 1984
P	V	5.0×10^{-8}	1.4×10^{-6}	5.0×10^{-8}	2.8×10^{-6}	1.0×10^{5}	Collier and Edmond, 1984
Pd	II			1.8×10^{-13}	6.6×10^{-13}	5.0×10^{4}	Lee, 1983
Pr	III	3.0×10^{-12}	5.0×10^{-12}	3.2×10^{-12}	7.3×10^{-12}		de Baar *et al.*, 1985; de Baar *et al.*, 1983
Pt	II	3.0×10^{-13}	3.0×10^{-13}	6.0×10^{-13}	1.4×10^{-12}		Hodge *et al.*, 1985; Donat and Bruland, 1995
Re		3.7×10^{-11}		5.5×10^{-11}			Donat and Bruland, 1995
Sc	III	1.4×10^{-11}	2.0×10^{-11}	8.0×10^{-12}	1.8×10^{-11}	5.0×10^{3}	Bruland, 1983
Se	IV	1.0×10^{-10}	9.0×10^{-10}	7.0×10^{-11}	9.0×10^{-10}	3.0×10^{4}	Cutter and Bruland, 1984; Measures *et al.*, 1984
Se	VI	5.0×10^{-10}	1.5×10^{-9}	1.3×10^{-10}	1.25×10^{-9}		Cutter and Bruland, 1984; Measures *et al.*, 1984

APPENDIX I (contd)

		Concentrations (M)					
		Atlantic		Pacific			
Element	Ox	surface	deep	surface	deep	T_y (yr)	Reference*
Si	IV	1.0×10^{-6}	3.0×10^{-5}	1.0×10^{-6}	1.5×10^{-4}	3.0×10^{4}	Collier and Edmond, 1984
Sm	III	2.7×10^{-12}	4.4×10^{-12}	2.7×10^{-12}	6.8×10^{-12}	200	de Baar *et al.*, 1985; Elderfield and Greaves, 1982
Sr	II	8.9×10^{-5}	9.0×10^{-5}	8.9×10^{-5}	9.0×10^{-5}	4.0×10^{6}	Bruland, 1983
Tb	III	7.0×10^{-13}	1.0×10^{-12}	5.0×10^{-13}	1.6×10^{-12}		de Baar *et al.*, 1985; de Baar *et al.*, 1983
Ti		4.5×10^{-11}	2.0×10^{-10}	6.0×10^{-12}	2.5×10^{-10}		Donat and Bruland, 1995
Tm	III	8.0×10^{-13}	1.0×10^{-12}	4.0×10^{-13}	2.0×10^{-12}		de Baar *et al.*, 1985; de Baar *et al.*, 1983
V	V	2.3×10^{-8}		3.2×10^{-8}	3.6×10^{-8}	5.0×10^{4}	Collier, 1984; Morris, 1975
W					6.0×10^{-10}		Donat and Bruland, 1985
Y	III				1.5×10^{-10}		Donat anad Bruland, 1995
Yb	III	3.0×10^{-12}	4.5×10^{-12}	2.2×10^{-12}	1.3×10^{-11}	400	de Baar *et al.*, 1985; Elderfield and Greaves, 1982
Zn	II	1.5×10^{-10}	1.6×10^{-9}	1.5×10^{-10}	8.2×10^{-9}	5.0×10^{3}	Donat and Bruland, 1995
Scavenged elements							
Al	III	3.7×10^{-8}	2.0×10^{-8}	5.0×10^{-9}	5.0×10^{-10}	150	Orians and Bruland, 1985
As	III			3.0×10^{-10}	7.0×10^{-11}		Andreae, 1979
Bi	III	2.5×10^{-13}		2.0×10^{-13}	2.0×10^{-14}		Lee, 1982
							Measures *et al.*, 1984
Ce	III	6.6×10^{-11}	1.9×10^{-11}	1.1×10^{-11}	4.0×10^{-12}	100	Elderfield and Greaves, 1982
							de Baar *et al.*, 1985
Co	II			1.2×10^{-10}	2.0×10^{-11}	40	Knauer *et al.*, 1982
		2.7×10^{-11}	1.5×10^{-11}	2.3×10^{-11}	1.5×10^{-11}		Donat and Bruland, 1995
Cr	III			2.0×10^{-10}	5.0×10^{-11}		Cranston, 1983
							Murray *et al.*, 1983
Hg	II	2.5×10^{-12}	2.5×10^{-12}	1.7×10^{-12}	1.7×10^{-12}		Dalziel and Yeats, 1985
							Gill and Fitzgerald, 1985
I	-I	2.0×10^{-7}	1.0×10^{-11}	9.0×10^{-11}	6.0×10^{-11}		Bruland, 1983
							Elderfield and Truesdale, 1980

APPENDIX I (contd)

		Concentrations (M)					
		Atlantic		Pacific			
Element	Ox	surface	deep	surface	deep	T_y (yr)	Reference*
In	III	2.7×10^{-12}	9.0×10^{-13}	1.1×10^{-12}			Donat and Bruland, 1995
Mn	II	1.9×10^{-9}	1.8×10^{-9}	1.9×10^{-9}	8.0×10^{-10}	50	Collier and Edmond, 1984
Pb	II	5.0×10^{-10}	2.0×10^{-11}	5.0×10^{-11}	5.0×10^{-12}	50	Bruland, 1983
Sn	IV	2.0×10^{-11}	5.0×10^{-12}				Byrd and Andreae, 1982
Te	VI	9.0×10^{-13}	4.0×10^{-13}	1.0×10^{-12}	4.0×10^{-13}		Lee and Edmond, 1985
Te	IV	4.0×10^{-13}	2.0×10^{-13}	5.0×10^{-13}	1.0×10^{-12}		Lee and Edmond, 1985

Ox, oxidation state; T_y, mean oceanic residence time (Whitfield, 1979).
*Data compiled from Donat and Bruland (1995) and Whitfield and Turner (1987). The references identify the sources of the figures quoted and are not always the papers describing the original measurements.

APPENDIX II

Inputs to the Ocean System*

	River		Hydrothermal		Aeolian	
Element	Moles/yr	% total	Moles/yr	% total	Moles/yr	% total
As	8.4×10^8	52	7.2×10^8	45	4.9×10^7	3
Cd	6.58×10^6	23		0	2.2×10^7	77
Cu	8.73×10^8	14	5.0×10^9	79	4.6×10^8	7
Fe	2.65×10^{10}	25	2.3×10^{10}	22	5.7×10^{10}	54
Ni	3.15×10^8	66		0	1.6×10^8	34
P	1.37×10^{11}	76	3.3×10^{10}	18	9.7×10^9	5
Pb	1.79×10^{10}	3	1.5×10^8	27	3.9×10^8	70
Zn	1.7×10^{10}	50	1.4×10^{10}	41	3.1×10^9	9
Mn	5.52×10^9	53	4.9×10^9	47		

*Global fluxes to the ocean (moles/yr) expressed as percentages of the total input flux.

APPENDIX III

Redfield Correlations for Trace Metals

A. Relationship between dissolved Cu and Si in the oceans.

$$Cu(\times 10^{-9}\,M) = aSi(\times 10^{-3}\,M) + b$$

Region	Latitude	Longitude	a	b	r	n	Depth range	Reference
Atlantic Ocean								
Sargasso Sea	~35.00° N	~64.5° W	0.049	1.02	0.78	21	0–3900	Jickells and Burton, 1988
Sargasso Sea	34.00° N	66.00° W	0.041	1.12	0.93	10	0–2962	Bruland and Franks, 1983
Sargasso Sea	35.39° N	71.53° W	0.044	0.82	0.9	11	500–3000	Hanson *et al.*, 1988; Si from Sakamoto-Arnold *et al.*, 1987
	33° N	20° W	0.023	0.67	0.88	21	0–4500	JGOFS, Nolting *et al.*, 1991
Southern Ocean								
Scotia Sea	57.00° S	49.00° W	0.024	0.59	0.91	19	100–4000	Nolting and de Baar, 1990
	60.46° S	63.26° W	0.012	0.84	0.96	10	0–1850	Martin *et al.*, 1990
Drake Passage	~50° S	~33° E		n/c				Orren and Monteiro, 1985
Marion Island	67.20° S	28° E		n/c				Orren and Monteiro, 1985
Indian Ocean								
NW Indian Ocean	~0–20.00° N	~68.00° E	0.024	0.73	0.94	15	150–3000	Danielsson, 1980
Upwelling zone	22.30° N	60.40° E	0.019	−0.13	0.95	9	250–3000	Saager *et al.*, 1992
Pacific Ocean								
	52.40° S	178.00° W	0.021	1.11	0.914	19	0–5257	Boyle *et al.*, 1977
	42.31° S	144.59° W	0.012	0.73	0.95	7	0–1000	Bruland, 1980
	50° N	145.00° W	0.006	1.56	0.9	16	0–1500	Martin *et al.*, 1990

Reproduced from: Nolting, R. F., de Baar, H. J. W., van Bennekon, A. J. and Masson, A. (1991). Cadmium, copper and iron in Scotia Sea, Weddell Sea and Weddell/Scotia confluence (Antarctica). *Marine Chemistry* **35**, 219–243; with the permission of the copyright holders, Elsevier Science.
n/c, not correlated.
a,b = regression parameters; r = correlation coefficient; n = number of data points.

APPENDIX III (contd)

B. Relationship between dissolved Zn and Si in the oceans

$$Zn(\times 10^{-9}\,M) = aSi(\times 10^{-6}\,M) + b$$

Region	Latitude	Longitude	a	b	r	n	Depth range	Reference
Atlantic Ocean								
	45.00–53.00° N	35.00–41.00° W	0.17	2.3	0.71	>50	0–4310	Yeats and Campbell, 1983
	34.06° N	66.07° W	0.09	0.27	0.84	10	0–2962	Bruland and Franks, 1983
Indian Ocean								
	All stations		0.06	0.5	0.89	50	0–4000	Saager *et al.*, 1992
	14.30° N	67.00° E	0.09	−0.33	0.91	12	175–2500	
	21.16° N	63.22° E	0.06	1.37	0.86	10	0–1200	
	22.30° N	60.40° E	0.05	1.31	0.92	17	0–3000	
	23.30° N	59.00° E	0.05	0.5	0.89	19	0–2750	
Pacific Ocean								
	37.05° N	123.22° W	0.06	−0.18	0.99	10	0–2950	Bruland *et al.*, 1978a
	32.00–37.00° N	122.00–144° W	0.05	0.02	1	>50	0–4875	Bruland, 1980

Reproduced from: Saager, P. M., de Baar, H. J. W. and Howland R. J. (1992). Cd, Zn, Ni and Cu in the Indian Ocean. *Deep-Sea Research* **39**, 9–35, with the permission of the copyright holders, Elsevier Science.

APPENDIX III (contd)

C. Relationship between Cd and PO_4 in the oceans[a]

$$Cd(\times 10^{-9}\,M) = aP(\times 10^{-6}\,M) + b$$

Region	Latitude	Longitude	a	b	r	n	Depth range	Reference
Arctic Ocean								
Arctic Atlantic	82.31° N	43.57° E	0.14	0.075		10	0–2400	Danielsson and Westerlund, 1983
Atlantic Ocean								
NW Atlantic	34.06° N	66.07° W	0.25	−0.019	1.00	11	0–2962	Bruland and Franks, 1983
NE Atlantic	48.30° N	20.00° W	0.2	−0.004	0.98	8	0–2700	Danielsson *et al.*, 1985
	33.00° N	20° W	0.19	0.005	0.98	16	0–4600	
Southern Ocean								
EPOS 159	57° S	49° W	0.63	−0.76	0.91	30	0–3931	Nolting *et al.*, 1991
EPOS 156	61° S	49° W	0.57	−0.62	0.76	17	0–2187	
EPOS 153	62° S	49° W	0.56	−0.71	0.87	11	0–1500	
Drake Passage	60.46° S	63.26° W	0.65	−0.85	0.92	10	0–1850	Martin *et al.*, 1990
Antarctic Indian	54.40° S	63.06° E	0.68		0.80	8	0–3346	Bourdin *et al.*, 1987
Antarctic Indian	61.05° S	66.20° E	0.82		0.78	8	0–3551	Bourdin *et al.*, 1987

Indian Ocean								
NW Indian	14.30° N	67.00° E	0.5	−0.49	0.97	14	100–4000	Saager *et al.*, 1992
	14.30° N	67.00° E	0.15	−0.054	1.00	4	0–100	
NW Indian	22.30° N	60.40° E	0.87	−1.26	0.96	10	50–3000	Saager *et al.*, 1992
Upwelling	22.30° E	60.40° E	0.16	−0.014	0.97	5	5–50	
South Pacific Ocean								
SW Pacific GEOSECS 293	52.50° S	178.05° W	0.581	−0.41	0.88	19	0–5271	Boyle *et al.*, 1976
Tasman Sea (shelf)	34.50° S	171.70° W	0.38	−0.001	0.98	11	0–866	Hunter and Ho, 1991
Tasman Basin	35.84° S	162.67° W	0.29	−0.05	0.95	33	0–1793	
Tasman Basin	37.16° S	166.13° E	0.25	0.14	0.91	10	150–1500	
North Pacific Ocean								
NE Pacific (three stations)	~35° N	~130° W	0.347	−0.068	0.99	34	0–5000	Bruland, 1980
NE Pacific	30.34° N	170.36° E	0.28	0.054	0.94	21	0–5446	Boyle *et al.*, 1976

[a]Compiled from Nolting *et al.* (1991) and Saager (1994).

APPENDIX III (contd)

D. Relationship between dissolved Ni, PO_4 and Si in the oceans[a]

$$Ni(\times 10^{-9}\,M) = aPO_4(\times 10^{-6}\,M) + bSi(\times 10^{-6}\,M) + c$$
$$\text{or } Ni(\times 10^{-9}\,M) = aPO_4(\times 10^{-6}\,M) + c$$
$$\text{or } Ni(\times 10^{-9}\,M) = bSi(\times 10^{-6}\,M) + c$$

Region	Latitude	Longitude	a	b	c	r	n	Depth range	Reference
Atlantic Ocean									
	10.00° S–60.00° N	36.00–0.00° W	4.2		1.52	0.34	>50	surface	Kremling, 1985
	40.00–59.00° N	15.00–20.00° W	1.44		1.94	0.94	23	0–2800	Danielsson *et al.*, 1985
	34.06° N	66.07° W	2.8		2	0.982	9	0–2962	Bruland and Franks, 1983
	45.00–53.00° N	35.00–41.00° W	1.5		2.2	0.886	65	0–4310	Yeats and Campbell, 1983
				0.063	3.3	0.684			(Si only)
			1.2	0.019	2.2	0.896			(Combined Si and PO_4)
Indian Ocean									
	2.43° N	66.02° E		0.048	5.2	0.92	19	0–3132	Danielsson, 1980
	19.01° N	67.01° E							
	All stations			0.054	2.45	0.944	>50	0–4000	Saager *et al.*, 1992
	14.30° N	67° E		0.048	2.5	0.949	16	0–4000	
	19.00° N	67° E		0.074	2.57	0.971	19	0–3200	
	21.16° N	63.22° E		0.05	3.03	0.95	17	0–3363	
	22.30° N	60.40° E		0.053	2.44	0.977	20	0–3000	
	23.30° N	59.00° E		0.064	1.91	0.987	20	0–2750	
Pacific Ocean									
	16.32° N	123.01° W	1.08	0.036	3.3	0.94	21	0–4187	Sclater *et al.*, 1976
				0.072	3.8	0.98	11	0–1200	With Si only
	31.23° N	150-02° W	0.98	0.03	3.7	0.94	12	0–4939	Sclater *et al.*, 1976
				0.043	4.4	0.957	12		With Si only
	177.2° N	53.7° W	0.38	0.02	4.8	–	16	0–3711	Boyle *et al.*, 1981
				0.027	4.7	0.905	16		With Si only
	32–37° N	122–144° W	0.95	0.033	2.74	0.995	>50	0–4875	Bruland, 1980
	175.5° N	52.4° W	1.88	0.009	2.5		19	0–5271	Boyle *et al.*, 1981

[a]Reproduced from: Saager, P. M., de Baar, H. J. W. and Howland, R. J. (1992). Cd, Zn, Ni and Cu in the Indian Ocean. *Deep-Sea Research* **39**, 9–35, with the permission of the copyright holders, Elsevier Science. a, b = regression parameters; c = constant; r = correlation coefficient; n = number of data points.

The Sea Cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): Its Biology and Exploitation as Beche-de-Mer

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One of the most intensively studied holothurians, Holothuria scabra *has been discussed in the literature since 1833. The species is important for several reasons: (1) it is abundant and widely distributed in shallow soft-bottom habitats throughout the Indo-Pacific; (2) it has a high value on the Asian markets, where it is mainly sold as beche-de-mer; and (3) it is the only tropical holothurian species that can currently be mass produced in hatcheries. Research on* H. scabra *continues but because of commercial exploitation, wild stocks are declining. This review compiles data from 14 theses and 352 technical reports and scientific papers pertaining to the biology, ecology, aquaculture and fisheries of* H. scabra. *Although several references are likely to have been missed by our investigation, we present the most complete reference list to date, including obscure material published by local institutions and/or in foreign languages. Our main aim was to summarize and critically discuss the abundant literature on this species, making it more readily accessible to all those wishing to conduct fundamental research, or aquaculture and stock enhancement programmes, on* H. scabra *across its entire geographic range.*

1. INTRODUCTION

Holothurians, commonly known as sea cucumbers, have been harvested for over 1000 years in the Indo-Pacific regions to supply markets in Asia for beche-de-mer, i.e. the dried body wall of the animal (Anonymous, 1975; Conand and Sloan, 1989; Conand, 1990; Conand and Byrne, 1993; D. B. James and P. S. B. R. James, 1994). The demand for beche-de-mer has been growing, especially with the re-entry of China into world trade during the 1980s. However, inadequate management of the sea cucumber fishery has resulted in severe overfishing in many countries, so that natural stocks are depleted almost everywhere within their geographic distribution (Preston, 1990a; Conand and Byrne, 1993; Holland, 1994a, b; Conand, 1998a; Battaglene, 1999a; Battaglene and Bell, 1999; Morgan 1999a; Battaglene *et al.*, in press). In addition to being exported, some species of sea cucumbers in Papua New Guinea, Samoa and Fiji, including *Holothuria scabra*, are also eaten locally (Shelley, 1985a; Conand, 1990; Adams, 1992; Conand and Byrne, 1993).

Although ca. 20 holothurian species are fished commercially around the world, only a few yield first grade beche-de-mer (Conand, 1989, 1990; Conand and Byrne, 1993; South Pacific Commision, 1994, 1995). The

sandfish *H. scabra* is one of these species and can fetch between ca. 50 and 100 US$ kg^{-1} dry weight as beche-de-mer (Conand, 1989; Mercier and Hamel, 1997). Interestingly, *H. scabra* has not always been so popular. Before commercial harvests bloomed in the 1970s, fishermen in Sri Lanka and other countries discarded *H. scabra* as an unclean animal and everyone who touched one would immediately wash their hands (Anonymous, 1978a). *H. scabra* fisheries have since become an important source of income for local fishermen in Indonesia, Papua New Guinea, India, Madagascar, Solomon Islands, Philippines and in many other Pacific and Indian Ocean countries (Conand and Sloan, 1989; Conand, 1990, 1998a; Conand and Byrne, 1993; Battaglene and Bell, 1999).

With the great demand for beche-de-mer and the response from local harvesters, the increasing harvest pressure on natural populations of *H. scabra* has created a severe crisis. Those wishing to restore depleted populations and to develop efficient aquaculture and stock enhancement programmes quickly encountered a lack of knowledge of most aspects of the biology and ecology of the species. The urgency of this situation has prompted many countries to conduct studies and rearing trials on *H. scabra* over the last decade, with the result that knowledge accumulated rapidly but has been inefficiently shared. Moreover, data were seldom assessed critically and results were often published in grey literature, if at all. The main problems encountered include doubtful identification of the species being studied and poor description of the methodologies used. Uncertainties and inexactitudes were widespread and many projects were duplicated, thus constraining the overall scientific progress in the field of *H. scabra* studies.

Since the first mention of *H. scabra* in the early 1800s, the species has been reported or discussed in hundreds of books, theses, reports, popular and scientific articles, of which D. B. James (1994a) made a partial list in his annotated bibliography on sea cucumbers. Some important contributions to the literature are often difficult to find or to consult, either because they were written in Malay, Indonesian or other languages, or because they were published in local journals or internal reports. This might explain why many researchers, such as Baskar (1994) and D. B. James (1994b), lamented a lack of knowledge of *H. scabra* in spite of the obvious interest and many ongoing research projects.

For this study, we have compiled and summarized the existing documents on the systematics, biology, ecology, culture and fisheries of *H. scabra* to make them readily accessible. This review should enable future research to focus more clearly on advancing our knowledge of this overfished species, which is both ecologically and commercially valuable throughout the Indo-Pacific.

2. SYSTEMATICS

Class **HOLOTHUROIDEA**
Order ASPIDOCHIROTIDA
Family Holothuriidae

This family of about 170 species is typically tropical (Rowe, 1969), although a few species occur in temperate waters. The holothuriids are known as fossils at least as far back as the early Jurassic (Gilliland, 1993).

Genus *Holothuria* Linnaeus, 1767

Diagnosis: Numerous locomotory tube feet on ventral surface of body, which is often flattened; dorsal surface often arched, with more or less conspicuous conical papillae. Calcareous ring stout; interradials about half as long as wide but not so wide as to be curved. Ossicle tables in some form almost always present; in addition buttons, rods, rosettes, perforated plates present or absent (after Rowe, 1969).

Remarks: *Holothuria* comprises approximately 120 species, most of which occur in shallow tropical or subtropical waters. These are usually large and conspicuous sea cucumbers, although a few species are secretive, living under rocks or burrowing into sandy substrata. Attempts to subdivide the genus (Panning, 1929, 1934a, b, 1935a, b; Deichmann, 1958) were only partially successful; they solved some problems and created others. In an excellent review, Rowe (1969) elaborated upon Deichmann's partial revision. The subgenera that Rowe diagnosed have been widely accepted.

Holothuria (Metriatyla) Rowe, 1969

Diagnosis: Size small to moderate, up to 200 mm in length; tentacles 20; feet arranged irregularly on flattened ventral "sole"; dorsal surface arched; papillae conspicuous, conical, irregularly arranged dorsally; an irregular fringe of marginal papillae sometimes present; body wall usually about 2 mm thick and gritty to touch; radial plates of calcareous ring up to three times as long as interradials; ossicles tables and buttons; tables with smooth disc and spire terminating in few to many small spines; buttons simple, with irregularly arranged knobs and 3–10 pairs of relatively large holes (after Rowe, 1969).

Type species: *Holothuria scabra* Jaeger, 1833

Remarks: Rowe (1969) included nine species in this subgenus; all are known only from the Indo-west-Pacific.

Holothuria (Metriatyla) scabra Jaeger, 1833

- *Holothuria scabra* Jaeger, 1833: 23; Panning, 1944: 67, figs 34–5.
- *Holothuria tigris* Selenka, 1867: 333.
- *Holothuria cadelli* Bell, 1887: 144.
- *Holothuria gallensis* Pearson, 1903: 203.
- *Holothuria (Holothuria) scabra* – Panning, 1934: 80, fig. 66a–f (includes synonymy prior to 1934).
- *Holothuria (Halodeima) scabra* – Mortensen, 1934: 6.
- *Holothuria (Metriatyla) scabra* – Rowe, 1969: 160, fig. 20; Cherbonnier, 1980: 647, fig. 16A–L (includes synonymy prior to 1980); 1988: 135, fig. 55A–O; Massin, 1999: 30, figs 22a–l, 23, 110f (includes synonymy prior to 1999).

Diagnosis: Tables with spires of moderate height, terminating in ca. 20 small spines. Disc of table with 8–12 holes. Buttons with 3–5 pairs of holes (after Rowe, 1969).

Remarks: The partial synonymy given above identifies publications in which complete synonymies and systematic descriptions can be found. Colour photographs of this species are provided in Féral and Cherbonnier (1986), and Massin (1999). Pearson's (1903) *Holothuria gallensis* was shown in a later paper (Pearson, 1910) to be synonymous with *H. scabra*. The vernacular names of *H. scabra* vary markedly across its geographic range (Table 1, p. 137).

Conand (1989, 1990, 1994) described a possible subspecies, *H. scabra versicolor*. This is discussed below in the general morphology section.

3. GEOGRAPHIC RANGE

Holothuria scabra is known from locations throughout the Indo-Pacific, roughly between latitudes 30° N and 30° S (Clark and Rowe, 1971; Conand, 1998b; Massin, 1999) (Figure 1). It has been reported from: western, northern and eastern Australia, including Cocos, Ashmore and Cartier Islands (Clark, 1931, 1938, 1946; Stephensen *et al.*, 1958; Endean, 1953, 1956, 1957; Gibbs *et al.*, 1976; Cannon and Silver, 1986; Marsh *et al.*, 1993; Marsh, 1994; Rowe and Gates, 1995; Morgan, 1996; Carter *et al.*, 1997; Massin, 1999), Cook Islands (Zoutendijk, 1989), Caroline Islands (D. L. Pawson, personal communication), China (Liao, 1980, 1984, 1997;

Figure 1 Geographic distribution of *Holothuria scabra*. (From Massin (1999) with permission.)

Anonymous, 1991; Liao and Clark, 1995), Democratic Republic of Yemen (Matthes, 1983; Amir, 1985; Gentle, 1985), Egypt (Mortensen, 1937; Din, 1986), Fiji (Preston, 1990a; Adams, 1992; Stewart, 1993), Guam (D. L. Pawson, personal communication), Hong Kong (Clark, 1980), India, including Andaman and Nicobar Islands (Satyamurti, 1976; D. B. James, 1978, 1982, 1986a, b, 1987a, 1988, 1989a, 1991, 1994b, c, d, e, f, 1995a; Parulekar, 1981; Soota *et al.*, 1983; Tikader and Das, 1985; Mukhopadhyay, 1988; P. S. B. R. James, 1990; Alagaraja, 1994; P. S. B. R. James and D. B. James, 1993, 1994a, b; D. B. James and P. S. B. R. James, 1994; Rengarajan and James, 1994; D. B. James, 1999), Indonesia (Sluiter, 1901; Daud and Mansyur, 1990; Daud, 1992; Hanafi *et al.*, 1992; Rachmansyah *et al.*, 1992; Daud *et al.*, 1993; Tangko *et al.*, 1993a; Rowe and Gates, 1995; Conand and Tuwo, 1996; Moore, 1998; Massin, 1999; Tuwo, 1999), Japan (Kobayashi *et al.*, 1991; Wiedemeyer, 1992), Kenya (Humphreys, 1981; Samyn, 2000), Kosrae (Kerr, 1994), Madagascar (Cherbonnier, 1988; Conand *et al.*, 1998), Malaysia (Ridzwan *et al.*, 1995; Biusing, 1997; Baine and Sze, 1999), Marshall Islands (D. L. Pawson, personal communication), Mozambique (Balinski, 1958; Macnae and Kalk, 1958, 1962; Rowe and Richmond, 1997; Abdula, 1998), New Caledonia (Cherbonnier, 1952, 1980; Féral and Cherbonnier, 1986; Conand, 1989, 1990, 1993, 1994; NPFD, 1993), Palau (Yamanouchi, 1939, 1956), Papua New Guinea (Clark, 1921; Shelley, 1981, 1985a, b; Anonymous, 1990; Lokani, 1990, 1995a, b, 1996; Lokani *et al.*, 1995a, b, 1996; Kare, 1996; Massin, 1999), Saudi Arabia (Clark, 1952; Tortonese, 1979; Price, 1982), the Seychelles (Clark, 1984), the Philippines (Semper, 1868; Domantay, 1934; Tan Tiu, 1981a, b; Reyes-Leonardo, 1984; Ong Che and Gomez, 1985; Reyes-Leonardo *et al.*, 1985; Leonardo and Cowan, 1993; Schoppe, 2000), the Solomon Islands (Adams *et al.*, 1994; Holland, 1994a, b; Richards *et al.*, 1994; Battaglene and Seymour, 1998; Battaglene and Bell, 1999; Hamel and Mercier, 1999), Sri Lanka (Pearson, 1903; Anonymous, 1978a, 1984; Elanganayagam *et al.*, 1981; Moiyadeen, 1994), Thailand (Wainiya, 1988; Bussarawit and Thongtham, 1999), Tonga (Sommerville, 1993; Anonymous, 1996a, b), Vanuatu (Chambers, 1989; Preston, 1993) and Vietnam (Serene, 1937; Dawydoff, 1952; Loi and Sach, 1963; Levin and Dao Tan Ho, 1989). Massin (1996, 1999) also indicated that *H. scabra* was found in Somalia, the Maldives, South Africa and Mauritius. Furthermore, it has been suggested that *H. scabra* is also present in Zanzibar, and most probably in several other countries or regions in both the Indian and Pacific Oceans (Theel, 1886; Sluiter, 1901; D. B. James, 1991; Sant, 1995) (Figure 1). Notably, the species has not yet been reported from Hawaii.

Table 1 lists the common and local names for *Holothuria scabra*.

Table 1 Common names of *Holothuria scabra* throughout its geographic distribution.

Locations	Common names	References
Worldwide	Sandfish	Baird, 1974; Gentle, 1979; Preston, 1993; Sakthivel and Swamy, 1994; South Pacific Commission, 1994; Conand, 1998b, 1999b
Burma	Pan-le-pet-kye, Pin-lehmyaw	Sakthivel and Swamy, 1994
China	Tok-sum, Tok-som, Puti-an, Thuk-su, Hai-som, Chalkyfish, Paishen, Peh-sim	Baird, 1974; Gentle, 1979; Conand, 1989, 1990; D. B. James, 1989a; Van Eys and Philipson, 1991; South Pacific Commission, 1994
Fiji	Dairo	Adams, 1992; Adams *et al.*, 1994; South Pacific Commission, 1994
Hong Kong	Sand sea cucumber, Hoy sum	Tiensongrusmee and Pontjoprawiro, 1988; Sakthivel and Swamy, 1994; South Pacific Commission, 1994
India	Vella Attai, Kadal Attai, Kadal Vellarikka, Patos, Dalamogon	D. B. James, 1989b; D. B. James and P. S. B. R. James, 1994; Sakthivel and Swamy, 1994; South Pacific Commission, 1994
Japan	Namako	Conand, 1989; Sakthivel and Swamy, 1994
Madagascar	Zanga fotsy, Bemavo, Tricot, Zanga mena	Conand, 1999b
Malaysia	Trepang, Putih, Tepuak	Sakthivel and Swamy, 1994; Biusing, 1997; Forbes *et al.*, 1999
New Caledonia	Holothurie de sable, sandfish	Conand, 1989
Palau	Rebotel	South Pacific Commission, 1994
Philippines	Rebothal, Patos, Dalamogon	Baird, 1974; Kanapathipillai and Sachithananthan, 1974; Trinidad-Roa, 1987
Samoa	Fugafuga ai	Anonymous, 1975; South Pacific Commission, 1994
Thailand	White sea cucumber	Bussarawit and Thongtham, 1999;
Yemen	Chalkyfish	Gentle, 1985

Table 2 Phenotypic and morphometric characteristics of *Holothuria scabra* over its geographic range.

Locations	Body wall colour	Size (mm)	Weight (g)	Body texture and thickness	Body wall morphology	Number of tentacles	References
General	Upper surface light grey, dull cream, olive brown, almost black also covered with fine black spots in the wrinkles, lower surface usually white	300	–	–	Short and stout flattened at the end	–	Baird, 1974; Conand, 1998a
Australia	Grey or black above with dark transverse wrinkles dorsally, grey or white below	–	–	–	Somewhat flattened, firm and pliable	–	Cannon and Silver, 1986
	–	200	–	Gritty, 1–5 mm	Flattened ventral surface, arched dorsally	20	Rowe, 1969
	Grey-white ventrally, grey or black with transverse wrinkles dorsally	–	–	–	–	–	Carter *et al.*, 1997
	Finely speckled grey with one large blotch on the dorsal side, ventral side white	170–300	–	Rough body surface	–	–	Clark, 1931
China	–	700	–	–	–	–	Anonymous, 1991
India	–	350	500	–	–	–	D. B. James, 1973
	Upper side grey with white or yellow horizontal bands, lower surface white with a number of fine black dots	400	500–1500	–	Body short and stout with blunt end and prominent wrinkles on dorsal surface	–	D. B. James and P. S. B. R. James, 1994
	–	300–350	500–600	–	–	–	D. B. James, 1973; Kanapathipillai and Sachithananthan, 1974; D. B. James *et al.*, 1994a
	–	300–500	500–1500	8–10 mm	–	–	Sachithananthan, 1986
	–	230	100–1400	–	–	–	Baskar, 1989, 1994
	Grey to black dorsally and white ventrally	100–400	25–2000	–	Robust, elongated and cylindrical with blunt ends	20	P. S. B. R. James, 1996

	Grey to black dorsally and white on the ventral surface	400	2000	10 mm thick	–	–	D. B. James, 1989b
	Dorsal surface grey to almost black with transverse yellow streaks	320	–	–	–	–	Gravely, 1927
	–	400	–	–	–	–	D. B. James, 1985
	–	100–400	25–2000	–	–	–	Jagadees and Sasidharan, 1990
	–	300–500	500–600	–	–	–	Anonymous, 1989
	Black upper side with white or light yellow bands across the body, lower side white in colour with black dots	400	–	–	–	–	D. B. James, 1989a; MPEDA, 1989
Indonesia	Grey dorsally with transversal greenish bands, grey-white ventrally	–	–	Rough skin, 2–3 mm thick	Body arched dorsally, more or less flat ventrally	20	Massin, 1999
Mozambique	Dorsally grey mottled with white and black above, white below	200	–	Skin very tough	–	–	Macnae and Kalk, 1958
New Caledonia	Grey to greenish, seldom black, ventral surface pale in colour	240	485	Gritty dorsal surface, 3–13 mm thick	Pronounced wrinkles dorsally	20	Conand, 1989, 1990; Féral and Cherbonnier, 1986
Papua New Guinea	From pale beige to dark brown, sometimes with dark spots dorsally, white or scattered with brown spots ventrally	–	–	–	–	–	Van den Spiegel *et al.*, 1992
Palau	Upper surface light grey, dull cream, olive brown or almost black, lower surface usually white	–	–	–	Short and stout and flattened at the ends	–	Kanapathipillai and Sachithananthan, 1974
Philippines	Light grey to greenish dorsally sometimes with dark grey or brown bands, white ventrally	160–280	–	–	Arched dorsally, flattened ventrally	–	Leonardo and Cowan, 1993
	–	100–200	–	Gritty and thin	Cylindrical, slightly arched dorsally, flattened ventrally	20	Tan Tiu, 1981b

4. GENERAL ANATOMY

4.1. Morphology

Table 2 summarizes some phenotypic and morphometric characteristics of *Holothuria scabra* throughout its geographic range. The body is robust, cylindrical, elongated, stout and flattened at the ends. Adults usually measure between 150 and 400 mm in length. The body wall accounts for about 56% of the total weight (Conand, 1989). The reported adult body weight varies considerably, between 500 and 2000 g, over its geographic range (Table 2). However, it has been noted that the weight depends on the amount of coelomic water and sediment in the alimentary canal (Conand, 1989; Baskar, 1994). The body wall is thick, gritty to the touch, and slimy. The dorsal side is convex and the ventral side is flat (D. B. James, 1989b). The mouth is oval, located antero-ventrally and encircled by 20 yellowish-grey peltate tentacles. The anus is postero-dorsal. The dorsal surface of the body wall is smooth with few thinly scattered tube feet and its colour is highly variable (Conand, 1989, 1998b, 1999a; Van den Spiegel *et al.*, 1992; Uthicke and Benzie, 1998) ranging from dark yellow to grey-brown and black, or intermixed, with irregular patterns of well-defined wrinkles (Table 2). Apart from the variability described later in the morphotype section, recent observations on specimens from several sites in the Indian Ocean show that the species from that region mostly presents characteristic elongated yellow spots, never observed in the Pacific (C. Conand, unpublished data). The ventral surface is white or cream; it is also rough, and bears numerous locomotory tube feet arranged irregularly (Table 2) (Tan Tiu, 1981b; Massin, 1999). Each dark spot on the ventral surface represents one tube foot (D. B. James, 1989b; Massin, 1999).

4.2. Internal anatomy

The calcareous ring comprises 10 plates, five radial and five interradial. There is a single very long stone canal, which is 12–15% of the body length (Massin, 1999). The tentacle ampullae are as long as the stone canal. The intestine is highly coiled and opens into the cloacal chamber (P. S. B. R. James, 1996). A single bunch of gonadal tubules is attached to the dorsal mesentery; the gonad opens to the exterior through a single gonopore in the mid-dorsal region near the anterior end (P. S. B. R. James, 1996).

Studies by Mary Bai (1971a, b, 1978, 1980, 1994) outline the internal anatomy of *H. scabra* in fine detail, including the digestive system, haemal system, respiratory tree, water vascular system and gonads. Mary Bai

(1980) indicates that the tentacles are covered by a thin cuticle, below which lies a dermis. Glandular cells with secretory granules are visible among the epithelial cells. Sensory fibres are observed among the connective tissue strands located below the dermis layer and a tentacular nerve is located beneath the nerve plexus. A lumen, called the tentacular canal, is bordered by a coelomic epithelium and filled with fluid and coelomocytes. The body wall is covered by a thin cuticle composed of dying cells from the epidermis. From the external surface, the successive layers of tissue that compose the body wall are a cuticle, an epithelium, a layer of glandular cells, a dermis containing the pigments and connective tissues, muscle strands, haemal lacunae, muscle layer and coelomic epithelium. *H. scabra* possesses five pairs of muscle bands running along the length of its body. The digestive system is composed of a mouth, an oesophagus, a stomach, a descending small intestine, an ascending small intestine, a large intestine, a cloaca and an anus. The digestive tract is supported by a mesentery (Mary Bai, 1980).

The haemal system in *H. scabra* consists of a ring, encircling the oesophagus, directly behind and closely attached to the water vascular ring. Two main sinuses, one dorsal and one ventral, run along the small intestine. From the haemal ring, five radial sinuses ascend along the aquapharyngeal bulb, accompanying the radial water canals. They run along the body wall and lie between the hyponeural sinus and radial water canals of the body wall. The dorsal and ventral haemal sinuses consist of an outer peritoneal epithelium, composed of columnar cells with deeply staining nuclei, a thick, uninterrupted circular muscle layer and a thick connective tissue with scattered amoebocytes. The *rete mirabile* consists of a thick peritoneal epithelium of elongated columnar cells, a thin circular muscle layer and connective tissue (Mary Bai, 1980).

The main organ involved in respiration in *H. scabra* is the respiratory tree, which originates from the anterior part of the cloaca near the junction with the large intestine and is attached to the body wall by irregular strands of connective tissue. It is divided into right and left arborescent tubes that extend anteriorly in the coelomic cavity up to the end of the aquapharyngeal bulb. The two main branches give rise to finer branches, which fill the entire coelomic cavity, surrounding the internal organs. The tubules are colourless, transparent and terminate in small thin-walled vesicles. The left tree is intermingled with the lacunar network of the *rete mirabile* of the ascending small intestine. Apart from these two main branches, there are two to three short, branched tubes originating from the base of the common stem of the respiratory tree. The cloaca of *H. scabra* pumps rhythmically in a motion called cloacal pumping. At this time the terminus of the digestive tract is closed. Exchange of gases also takes place through the tube feet and the integument (Mary Bai, 1980).

The coelom is filled with coelomic fluid, which is circulated by the cilia of peritoneal epithelium. It is less alkaline than sea water and contains a variety of free cells known as coelomocytes. The coelomocytes occur in the haemal fluid and the fluid of all the coelomic compartments except the hyponeural sinuses. The coelomocytes comprise: lymphocytes, which are small spherical cells of ca. 4–6 μm in diameter, phagocytes ranging between 4 and 20 μm; morula cells that form aggregations, each cell measuring between 3 and 12 μm; haemocytes measuring 2–6 μm; fusiform cells measuring 4–6 μm in length and crystal cells that measure 6–9 μm in diameter. The fluid in the water vascular system contains all types of coelomocytes that occur in the body fluid, but the lymphocytes and phagocytes are more common. The haemal system also contains a large number of floating morula cells. The abundance, variety and inclusions of the coelomocytes suggest that they serve vital roles in nutrition, transport of wastes and phagocytosis (Mary Bai, 1980).

The water vascular system consists of a circular ring canal or water ring, Polian vesicle and stone canal. The Polian vesicle is an ovoid or elongated sac 1.0–2.5 cm long, and it arises from the left ventral part of the ring canal. Mary Bai (1980) indicated that there is usually one, and very rarely two Polian vesicles, and Massin (1999) reported that *H. scabra* possessed only one Polian vesicle. Conversely, P. S. B. R. James (1996) refers to two or three Polian vesicles. The stone canal arises from the right dorsal part of the ring canal and floats freely in the coelom. It is 1–5 cm long and the wall of the stone canal is distended with folds (Mary Bai, 1980).

The nervous system consists of networks concentrated into ganglionated radial nerve cords and divided into ectoneural, deeper-lying hyponeural and entoneural systems (Mary Bai, 1980).

Indap *et al.* (1996) studied the effect of the "Cuvierian tubules" of *H. scabra* on a variety of potential predators. The work of Mary Bai (1971a, 1978, 1980), Leonardo and Cowan (1993), Soota *et al.* (1983) and Massin (1999) shows that this species does not possess Cuvierian tubules. Indap *et al.* (1996) studied either a different species or misidentified an internal organ.

4.3. Auto-evisceration and regeneration

Semper (1868) was the first to discuss the regenerative capabilities of *H. scabra*. Silver (1985) later described the autotomy area of the oesophagus and the mechanism of auto-evisceration. The autotomy area is characterized by a cell mass of early-stage lymphocyte-like cells in the connective tissue layer. However, Mary Bai (1971b) indicated that evisceration in *H. scabra* does not occur in nature, either spontaneously or seasonally.

Nonetheless, one eviscerated *H. scabra* was observed in the Solomon Islands during a field survey (J.-F. Hamel, unpublished data). Conand (1989) noted that 14.6% of *H. scabra* eviscerated during tagging procedures prior to field monitoring, a percentage significantly higher than the 1.1% observed in *H. scabra versicolor*. Without giving any details, Sachithananthan (1986), Leonardo and Cowan (1993) and Mary Bai (1994) observed that auto-evisceration in *H. scabra* was induced by collection and chemicals. Battaglene *et al.* (in press) stated that evisceration occurred in 4.5% of individuals during transport to the laboratory. Approximately 2 months are needed for *H. scabra* to regenerate its internal organs after evisceration (Cannon and Silver, 1986). Mary Bai (1971a, b, 1994) reported that feeding started again 7 days after evisceration, although the alimentary canal was fully restored only after 13–35 days. The respiratory tree is regenerated in about 19 days and the haemal system in 35 days (Mary Bai, 1971a, b).

4.4. Ossicles

Most studies of ossicles in *H. scabra* (Figure 2) have been made on adults (Gravely, 1927; Rowe, 1969; D. B. James, 1973, 1976; Mary Bai, 1980; Tan Tiu, 1981b; Soota *et al.*, 1983; Cherbonnier and Féral, 1984; Cannon and Silver, 1986; Wainiya, 1988; Van den Spiegel *et al.*, 1992; Conand, 1998b; Massin, 1999) except for a note describing the ossicles of a 30 mm long specimen in India (D. B. James, 1976). Cherbonnier (1955, 1988), Cherbonnier and Féral (1984), Van den Spiegel *et al.* (1992) and Massin (1999) showed that the ossicles of *H. scabra* varied little throughout its geographic range. Massin *et al.* (2000) described ossicle changes from pentactula through juveniles and adult specimens. This precise description can be used to identify juveniles collected in the field. In general, the ossicles consist of tables and knobbed buttons (Massin *et al.*, 2000). Ossicles of *H. scabra* vary mainly in early juveniles between 0.9 and 15 mm long. While ossicles are not observed in auricularia and doliolaria larvae, which instead possess elastic balls, ossicles are present in late pentactulae. Specimens 0.9–1.5 mm long have tables with a tall spire (4–5 cross-beams), no buttons, and large irregular perforated plates. Specimens 5–6 mm long have tables with a moderate spire (2–4 cross-beams) and a few smooth buttons. Specimens 9–16 mm long have tables with a low spire (1–2 cross-beams) and knobbed buttons. From 30 mm, ossicles are similar to those of adults, with more buttons and fewer tables (Figure 2). Several features of the ossicles of early juveniles, including their size, shape and prevalence, are unique to the species. Comparison with juveniles of other holothurian species indicates that presence of tables with a tall spire and

Figure 2 Ossicles of adult *Holothuria scabra*: (a) calcareous ring (IR, interradial piece; R, radial piece); (b) tables from dorsal body wall; (c) large table from dorsal body wall; (d) buttons from dorsal body wall; (e) large buttons from dorsal body wall; (f) rods from dorsal body wall; (g) table from ventral body wall; (h) buttons from ventral body wall; (j) tube foot buttons; (k) perforated rods from tube feet; (l) tentacle rods. (From Massin (1999) with permission.)

absence of buttons are plesiomorphic characters in the evolution of the Holothuriidae (Massin *et al.*, 2000).

4.5. Morphotypes observed

Important variations in the morphological and phenotypic appearance of *H. scabra* are observed throughout its geographic range (Table 2), and there is a high degree of polymorphism (Conand, 1990). Two main explanations for this have been proposed: (1) identification errors and (2) important morphological plasticity over the geographic range.

In the Pacific, *H. scabra* generally exhibit the characteristics of the type specimen, having a whitish to dark brown bivium occasionally with dark or black transverse markings (Cherbonnier and Féral, 1984; Féral and Cherbonnier, 1986; Conand, 1998b; Massin, 1999). Mercier *et al.* (1999a, b, 2000b, in press a) and Uthicke and Benzie (1998, 1999, in press a) also observed black body wall phenotypes within *H. scabra* populations in the Solomon Islands and Australia, respectively (Figure 3). Uthicke and Benzie (1998) found that both colour morphs were present at all depths. However, the proportion of black versus grey forms varies considerably among sites. Uthicke and Benzie (1998, 1999, in press a) did not find any

Figure 3 Grey and black forms of adult *Holothuria scabra* from Solomon Islands. Individuals ca. 25 cm long. (Photograph courtesy of S. C. Battaglene, ICLARM.)

genomic differences, and Mercier *et al.* (1999a) did not find any behavioural differences between the colour morphs. The possibility that young black *H. scabra* could be *H. scabra versicolor* has never been substantiated (Uthicke and Benzie, 1998, 1999). The hypothesis is further refuted by the fact that some black juveniles were commonly reared from the spawning of typically coloured adults (S. C. Battaglene, unpublished data). The Indian Ocean morphotype differs from the above-mentioned one by the presence of the yellow elongated dots (Sachithananthan, 1994a; Conand, 1999b), a characteristic that deserves more attention.

On the other hand, the variety *H. scabra versicolor* (Conand, 1989, 1990, 1998b, 1999b) presents the same characteristic variability in both oceans. The colour varies from cream to black with some mottled individuals. In New Caledonia, their respective percentages are 42% for cream, 34% for black and 24% for mottled.

In addition to the variability in colour and size evident in Table 2, behaviour and reproductive patterns, which will be discussed later, also differ considerably, suggesting that not all studies were conducted on *H. scabra*.

4.6. A possible subspecies

Conand (1990) was the first to identify a variety and possible subspecies of *H. scabra* from New Caledonia, called *H. scabra versicolor* (golden sandfish). The latter differs by its morphology, ecology and reproductive biology from the former (Table 3, Figure 4), but the spiculation, the calcareous peripharyngeal ring and the internal anatomy are very similar and the possibility of a new species has yet to be looked at in more detail, as noted by Conand (1990) and Massin (1999). The papillae and the tube feet of *H. scabra* are more developed than those of *H. scabra versicolor*. Furthermore, *H. scabra versicolor* does not possess dorsal surface wrinkles (Conand, 1989) (Figure 4). But the most striking difference is believed to reside in the size and weight of adults, *H. scabra versicolor* being larger and heavier with mean length and weight of 30–48 cm and 1.5 kg (Conand, 1989). While *H. scabra* is typically smaller in New Caledonia (Conand, 1989), similar and even larger sizes than those proposed for the *versicolor* variety have been reported for *H. scabra* in a few regions (Table 2). However, such comparisons are difficult because different investigators may have used different protocols to measure length and weight. For instance, D. B. James (1989b), P. S. B. R. James (1996) and Jagadees and Sasidharan (1990) mentioned weights up to 2000 g for lengths of 100–400 mm (Table 2), suggesting that they did not measure the contracted length and the weight without water and intestinal contents.

Table 3 Comparison between *Holothuria scabra* and *H. scabra versicolor* in New Caledonia.

Characters	*Holothuria scabra*	*Holothuria scabra versicolor*
Wrinkles	Present dorsally	Absent
Dorsal colour	Grey to black with green stripes	Clear beige to black or with large dark spots
Size (mm)	120–390	180–480
Weight (g)	50–1400	100–2800
Spawning	Biannual cycle	Once a year
Evisceration (%)	Common (10%)	Rare (1%)
Oocyte diameter (μm)	190	210
Size at first maturity (mm)	160	220
Burrowing cycle	Yes	Yes
Body wall thickness (mm)	6	9
Habitat	Protected lagoon, bay with terrigenous influence close to the shore and in the littoral, frequently in seagrass	Rarely found in the littoral, prefers terrigenous areas, found off the coast to 10 km
Substratum	Muddy sand	Muddy sand
Depth (m)	To 5 m	To 25 m
Ossicles	Abundant	Abundant
Density (no. 100 m^{-2})	6.83	0.82
Biomass (g 100 m^{-2})	2416	977
Quality of beche-de-mer	First grade	First grade

From Conand, 1989, 1990, 1993, 1994.

Using the latter measures, Mercier *et al.* (1999a, c, 2000b) found that the largest *H. scabra* in the Solomon Islands were consistently smaller than *H. scabra versicolor* described by Conand (1989, 1990, 1998b) who further mentioned that juveniles of both species can clearly be identified. The reproductive cycle is also different; *H. scabra versicolor* reproduces only once a year, and the gonad is heavier, with longer and thicker gonadal tubules (Conand, 1989, 1990). Further details on the ecology, biology and fisheries of this variety have been provided by Rasolofonirina (1997). Finally, while both *H. scabra* and *H. scabra versicolor* prefer to burrow in sandy substrata, the latter is usually found in deeper water around 25 m, and farther offshore, as far as 10 km from the coast (Conand, 1989, 1990, 1999a). It is also worth noting that fishermen give different local names to *H. scabra* and *H. scabra versicolor;* in Madagascar for example they are well distinguished in the north and the south-east where they are fished (Conand, 1999b).

Figure 4 (A) *Holothuria scabra* (ca. 25 cm long); (B) *H. scabra versicolor* (ca. 35 cm long). (Photographs by C. Conand.)

5. SPATIAL DISTRIBUTION, POPULATION STRUCTURE AND DYNAMICS

5.1. General habitat

Habitat characteristics of *Holothuria scabra* are presented in Table 4. Domantay (1936), Intes and Menou (1979), Levin (1979), Shelley (1981, 1985b), Conand (1989, 1990), Lokani *et al.* (1996) and Mercier *et al.* (2000a, 2000b) found that *H. scabra* were distributed mainly in low energy environments behind fringing reefs or within protected bays and shores. Only a few contradictory observations placed *H. scabra* in high-energy environments (Anonymous, 1991, 1996c), although the methods used to characterize the habitats were not provided. Numerous reports indicated that *H. scabra* are often found in areas of low salinity in India (D. B. James, 1989b; D. B. James and P. S. B. R. James, 1994; Jagadees and Sasidharan, 1990) and in Australia (Springhall and Dingle, 1967) but, unfortunately, no precise values of salinity were given. The recent work of Mercier *et al.* (1999a, b) demonstrated the ability of *H. scabra* to tolerate salinity decreases down to 20 p.s.u. by burrowing into the sediment, which could explain why this species can sometimes be found near estuaries. *H. scabra* is found in habitats with terrigenous inputs in New Caledonia (Conand, 1990, 1994), Papua New Guinea and Australia (Long and Skewes, 1997), in nutrient-rich environments in the Solomon Islands (Battaglene, 1999b; Mercier *et al.*, 2000b), and close to mangrove swamps in Australia (Stephensen *et al.*, 1958), Madagascar (Conand, 1997a), and the Solomon Islands (Mercier *et al.*, 1999c, 2000b).

H. scabra is one of the rare tropical species that prefer ordinary coastal areas to coral reefs (Conand, 1989; Uthicke and Benzie, 1999). A few studies indicate that *H. scabra* are attracted to muddy sand habitats (Shelley, 1981; Mercier *et al.*, 1999a) or mud (Baskar, 1994) (Table 2). According to laboratory experiments and field studies, juveniles show a clear preference for medium-sized grains around 0.4 mm and, to a lesser degree, for finer muddy sand, whereas coarse sand and crushed coral are avoided (Mercier *et al.*, 1999a). Associations with seagrass were observed in Papua New Guinea (Long and Skewes, 1997; Long *et al.*, 1996) and Australia (Vail, 1989; Uthicke and Benzie, 1999). Large populations were found near or within *Thalassia hemprichii* and *Enhalus acoroides* beds in the Solomon Islands (Mercier *et al.*, 2000b), within *Zostera* beds in Australia (Endean, 1953) and within *Enhalus* beds in Indonesia (Sipahutar and Soeharmoko, 1989).

Table 4 Habitat characteristics of *Holothuria scabra* over its geographic range.

Location	Age	Substratum	Energy	Salinity	Depth	Comments	References
General South Pacific	Adult	Silty sand or mud	Low	Estuarine	2–10 m	–	Baird, 1974; South Pacific Commission, 1994
Australia	Adult	–	–	Estuarine	–	–	Morgan, 1996
	Adult	–	–	–	0–10 m	Inshore	Rowe and Gates, 1995
	Adult	Sand	–	–	–	Reefs and seagrass beds	Cannon and Silver, 1986
	Adult	–	–	–	–	Close to mangrove	Stephenson *et al.*, 1958
	Adult	–	–	–	–	In *Zostera* flats	Endean, 1953
	Adult	Mud flats	–	–	>2 m (18 m)	Intertidal seagrass beds, deep bay	Uthicke and Benzie, 1999, in press a
	Juvenile	–	–	–	<2 m	In seagrass beds	Vail, 1989
China	Adult	Muddy sand	High	Coastal reef	–	Coastal reefs and where seaweeds are lush	Anonymous, 1991
India	Adult	Mud	–	–	–	–	Baskar, 1994
	Adult	Silty sand	–	Brackish waters	1–10 m	Near estuaries, on *Cymodocea* beds	D. B. James, 1989b
	Adult	Coral sand	–	–	2–10 m	Estuarine waters near rivers	Kanapathipillai and Sachithananthan, 1974
	Adult	Mud	–	Low salinity	Intertidal to 20 m	–	D. B. James *et al.*, 1994a
	Adult	Silty sand	–	Low salinity	Intertidal to 10 m	On *Cymodocea* beds	D. B. James and P. S. B. R. James, 1994
	Juvenile	Sand and mud	–	–	–	Among algae	D. B. James, 1976
	Adult	Mud and sand	–	–	Intertidal to 20 m	Mud and sand flats	D. B. James, 1994b
	Adult	Mud and sand	–	–	10–15 m	Seagrass bed and near mangrove or estuaries	Sachithananthan, 1986
	Juvenile	–	–	–	Near shore	–	Sachithananthan, 1986
	Adult	Mud and sand	–	Brackish	Shallow waters	–	Jugadees and Sasidharan, 1990
Indonesia	Adult	Sandy clay	–	–	–	Seagrass *Enhalus*	Sipahutar and Soeharmoko, 1989
Japan	Adult	Mud and sand	–	–	–	–	Wiedemeyer, 1992
Kosrae	Adult	–	–	–	Shallow water	–	Kerr, 1994
Malaysia	Adult	Sandy shore	–	–	10–40 m	Reef slope and rise	Baine and Sze, 1999
Mozambique	Adult	Sand	High	–	Intertidal	Across seagrass and algae	Anonymous, 1996c
	Adult	Sand and muddy sand	Low	33–35	–	Mangrove and seagrass areas	Macnae and Kalk, 1962

New Caledonia	Adult	Muddy sand	Low	–	0–5 m	Terrigenous input (<3.5 km from the coast)	Conand, 1989, 1994
	Adult	–	–	–	–	Reef flat, terrigenous input	Conand, 1990
	Adult	–	Low	–	0–12 m	Behind fringing reef	Intes and Menou, 1979
	Juvenile	–	Low	–	Intertidal	Near estuary flat	Conand and Tuwo, 1996
	Adult	Mud and sand	Low	–	0–5 m	Inner reef flats, fringing or islet reefs, terrigenous input	Conand, 1990
	Adult	Mud and sand	–	–	Rarely >5 m	Bay, close to mangrove and sandy beach	Conand, 1989, 1994
Palau	Adult	Sand flat	Low	–	Shallow	Close to reef margin along fringing reef, seagrass	Yamanouchi, 1939
Papua New Guinea	Adult	–	Low	Brackish	Shallow	Close to mangrove and behind fringing reef	Shelley, 1985b
	Adult	–	–	–	–	Seagrass with terrigenous sediments	Long and Skewes, 1997
	Adult	Silt	–	–	6–11 m	Seagrass bed	Van den Spiegel *et al.*, 1992
	Adult	–	–	–	–	Seagrass bed	Long *et al.*, 1996
	Adult	Mud, sand	–	–	LWNT to LWST	Inner reef flats	Shelley, 1981
Philippines	Adult	Sand	–	–	–	Shallow water, seagrass, algae beds, near estuary	Tan Tiu, 1981a
	Adult	Sand, coral rubbles	–	–	–	–	Leonardo and Cowan, 1993
	Adult	–	Low	–	–	Behind fringing reef	Domantay, 1936
Solomon Islands	Adult	Sand to mud	Low	Brackish water	<5 m	Protected inner-reef flats, bays and estuaries, near mangroves and terrigenous inputs	Battaglene and Bell, 1999
	Adult	Mud and sand	Low	30–33	Intertidal to 5 m	Close to mangrove and seagrass beds	Mercier *et al.*, 2000b
	Juvenile	Mud and sand	Low	30–33	<1 m	In seagrass beds	Mercier *et al.*, 2000b
Sri Lanka	Adult	–	–	–	6–20 m	–	Anonymous, 1978a
Yemen	Adult	Fine silty sand	Low	–	–	Shallow sheltered lagoon and turtlegrasses (*Halodule* and *Halophila*)	Gentle, 1985

LWNT, lower water neap tides; LWST, lower water spring tides.

5.2. Densities

In New Caledonia, *H. scabra* are found in habitats ranging from intertidal flats to depths of 10 m with muddy sand to sandy mud sediments. The density of *H. scabra* in New Caledonia ranges from 0.1 to 60 individuals per 100 m^2 with a mean of 6.8, whereas the mean biomass is 2.4 kg 100 m^{-2} (Conand, 1989). *H. scabra* ranked third in density and second in biomass among the 50 aspidochirotid holothurians studied by Conand (1989) in New Caledonia.

The density of *H. scabra* in the Solomon Islands was greater on mud (2.2 individuals per 100 m^2), muddy sand (1.0 individual per 100 m^2) and sand (0.8 individual per 100 m^2) than on silt, coral pebbles and seagrass beds (<0.20 individual per 100 m^2) (Mercier *et al.*, 2000b). Individuals <100 mm are mainly concentrated on muddy sand (34.7 individuals per 100 m^2) and mud (26.8 individuals per 100 m^2), although data were more variable and based on rough estimates from examination of ca. 3% of each substratum type (Mercier *et al.*, 2000b). In the Solomon Islands, the highest density measured, pooling all size classes together, is 34 individuals on 10 m^2 of muddy substratum containing between 5 and 10% of organic matter, at a depth of ca. 45 cm in an area that was never exposed at low tide (Mercier *et al.*, 1999c, 2000b).

Abundance of *H. scabra* is quite uniform throughout the Pacific and Indian Oceans with average values ranging between 0.2 and 0.6 individual per m^2 (Table 5). The highest density, aside from the above-mentioned data from the Solomon Islands and New Caledonia, was found by

Table 5 Density and biomass of *Holothuria scabra* over its geographic range.

Locations	Density (individuals m^{-2})	Biomass ($g\,m^{-2}$)	References
India (juvenile)	0.4–2.0	–	D. B. James, 1994b
Indonesia	0.0025–0.39	0.03–1.97	Mangawe and Daud, 1988
New Caledonia	0.068* (maximum of 0.6)	24	Conand, 1989, 1990, 1994
Papua New Guinea	0.29–1.35	–	Shelley, 1981, 1985a, b
	0.01–0.02	–	Lokani *et al.*, 1995b
	0.00–0.26	–	Lokani *et al.*, 1996
Solomon Islands	0.0075* all substrata combined; maximum 0.35 on muddy sand substrata	–	Mercier *et al.*, 2000b

*Average values.

D. B. James (1994b) with ca. 2 individuals per m^2 in India. Moreover, Amir (1985) indicated that *H. scabra* is the most abundant species of sea cucumber along the coast of Yemen.

Shelley (1985b) noted that *H. scabra* in Papua New Guinea had an annual production of $487\,kg\,ha^{-1}\,yr^{-1}$ considering the dry weight and $24\,kg\,ha^{-1}\,yr^{-1}$ for beche-de-mer.

5.3. Distribution and size structure

In the Solomon Islands, *H. scabra* is distributed according to a depth gradient coupled with the effects of granulometry and organic matter content of the sediment (Mercier *et al.*, 1999c, 2000b, in press a). The largest individuals (>250 mm), are located mainly in the deeper zone, >120 cm, on a sandy substratum. Animals ranging from 150 to 250 mm in length occur mainly around seagrass beds, on mud or muddy sand substrata in water 30–120 cm deep. Intermediate-sized individuals ranging from 40 to 150 mm are found on mud and muddy sand in shallow water fringing the intertidal zone and in the intertidal zone itself. The individuals distributed on the exposed portion of the substratum at low tide are buried and the small depressions they create retain water. *H. scabra* are not found exposed directly to the air. Similarly, P. S. B. R. James and D. B. James (1994a) observed that juveniles bury during low tide in Andaman Islands, although D. B. James (1994b) found that small individuals between 50 and 90 mm in length do occur on the substratum during low tide. The smallest *H. scabra* (10–40 mm) observed by Mercier *et al.* (2000b) were usually found on mud and muddy sand substrata, sometimes inside the seagrass beds, in 20–120 cm of water.

Most *H. scabra* individuals occur in areas of 5–10% organic matter, although the largest individuals appear to prefer those areas with <5% organic matter, and some small and medium-sized individuals can be found on substrata with >10% of organic content (Mercier *et al.*, 1999c, 2000b, in press a).

The size-frequency distribution of *H. scabra* in the Solomon Islands varied from one site to another (Mercier *et al.*, 2000b). Multiple cohorts were detected in the population of an unfished bay called Kogu Veke with sizes varying from >10 mm to ca. 330 mm. Conversely, only 45 individuals between 100 and 330 mm were collected at Kogu Halingi, a harvested site, showing a population structure without recent recruitment. Thirty-five intermediate-sized individuals, 120–280 mm long, also dominated collections at another fished site (Malmaragiri Inlet), with only three specimens <100 mm found in October 1997, and seven found in February 1998.

In New Caledonia, where the populations are mostly intertidal, Conand (1989, 1990, 1994) showed that size distribution was plurimodal, with poorly defined modes composed of individuals ranging from 120 to 360 mm length, with a general mean mode around 240 mm and no smaller individuals.

In India, Baskar (1994) indicated that the frequency distribution of *H. scabra* had a single mode, with the smallest individuals measuring 90 mm long and the largest 370 mm, with an average of 230 mm. D. B. James (1994b) noted that specimens 300–350 mm in length were found at between 5 and 10 m depth in the Gulf of Mannar, India.

A similar size-frequency distribution was observed in Papua New Guinea by Lokani *et al.* (1995b) and Lokani (1996). Lokani *et al.* (1996) further indicated that the size distribution was unimodal or bimodal, depending on the site studied in Papua New Guinea.

In Australia, along the Queensland coast, Uthicke and Benzie (1998, 1999) observed that the size-frequency distribution of *H. scabra* was unimodal. The average size from the three shallow populations studied was 98–178 mm, while it was much larger (269 mm) for a deeper trawled population.

5.4. Juveniles

Until recently, there were very few data on the habitat of *H. scabra* juveniles, which were rarely observed in the wild. Mercier *et al.* (2000b) described the distribution of juveniles of all sizes >10 mm on a muddy sand substratum near a seagrass bed in the Solomon Islands. A few intermediate-sized juveniles have also been found on fine sand on an inner reef flat exposed at low water in Papua New Guinea (Shelley, 1985a, b; Long and Skewes, 1997). Conand and Tuwo (1996) observed specimens of *H. scabra* about 100 mm long in the intertidal zone near an estuary flat in South Sulawesi. Gravely (1927) found a 50 mm long juvenile in India, and a 30 mm long juvenile *H. scabra* was discovered in India among algae by D. B. James (1976). D. B. James (1983) also found juveniles 60–160 mm long along the coast of India. D. B. James (1989b, 1994b, g) and P. S. B. R. James (1996) noted that 500 juveniles ranging from 65 to 165 mm were collected from the Andaman Islands in the intertidal region during low tide. Conand (1997a) observed an artisanal fishery exploiting juveniles in the north of Madagascar. Specimens were harvested from a *Syringodium* seagrass flat in front of a mangrove, and measured only 40–80 mm. They were heavily collected by women; the estimated CPUE (catch per unit effort) has been as high as 300 individuals per

woman per hour, but had already decreased markedly by the time of the study.

Lokani *et al.* (1995b) found that young individuals were absent from sites supporting adult *H. scabra*. This was not corroborated by Mercier *et al.* (1999c, 2000b, in press a) who found newly settled and small individuals in the same general area as adults in the Solomon Islands.

5.5. Movement and tagging

H. scabra move with the help of tube feet densely distributed on the ventral surface of the body wall and also through muscular action of the body wall. They are able to climb on hard surfaces such as rocks (D. B. James, 1989b). Conand (1983, 1989, 1994) tested the use of floy tags (e.g. t-shaped fasteners similar to those used in the clothing industry) for long-term mark-recapture experiments in New Caledonia. Although some individuals eviscerated, most tagged individuals were not affected by the procedure. However, this method of tagging did not prove to be very effective during long-term tracking (Conand, 1989, 1991). Stewart (1993) and Mercier *et al.* (2000b) found that numbers scratched on the body wall had no deleterious effects and remained visible for about 2 weeks, thus providing a valuable technique for short-term monitoring of displacement and speed. Using this method, Mercier *et al.* (1999a, b, c, 2000b, in press a) noted that young *H. scabra* moved 50–331 cm d^{-1} in the laboratory and 41–80 cm d^{-1} in the field. Lokani *et al.* (1995a, b, 1996) and Lokani (1996) observed that *H. scabra* moved at a mean speed of 12 cm min^{-1} and that while their movement was random, they could target a precise site. Mercier *et al.* (1999a, b) observed a locomotory speed of juvenile *H. scabra* of 3.6–15.4 mm min^{-1} depending on the size of the individuals. The locomotory speed of *H. scabra* was greater on non-optimal substrata such as crushed coral than on sand or muddy sand (Mercier *et al.*, 2000b).

Young *H. scabra* released in the field in three different habitats (sand, crushed coral, seagrass bed) exhibited apparently random movement, changing direction every day (Mercier *et al.*, 2000b). In general, the larger the sea cucumber, the greater distance travelled, except in a seagrass bed. In all habitats, individual migrations remained relatively constant over a 2 mo study period; however, there was a significant difference in distance travelled when the three habitats were compared both between comparable size classes and across size classes. The greatest displacement occurred on a mix of shells and crushed coral, with ca. 80 cm d^{-1}. The seagrass habitat induced the second greatest movement (ca. 51 cm d^{-1}) and the sand substratum induced the smallest movement (ca. 41 cm d^{-1}). Movements with respect to organic matter content of the substratum in the

three habitats were less conclusive and the highest mobility was recorded on the sediment of intermediate richness (Mercier *et al.*, 2000b).

In the laboratory, juveniles moved faster on bare surfaces or organically poor substrata than on rich substrata, indicating that they do indeed move around in search of food if organic matter is distributed unequally (Mercier *et al.*, 1999a). Calculations of the mean speed over the active period gave approximate values of 71–331 cm d^{-1} on a poor substratum, depending on the size of the juveniles. The same estimation yielded values of 50–215 cm d^{-1} on a rich substratum. Considering that faecal pellets are emitted less frequently on the poor substratum, the rapid movement of juveniles in such environments does not appear to be associated with higher feeding rates. Instead, the animals might spend a great deal of time wandering without feeding, suggesting that the energetic cost of processing poor sediment is higher than the cost of moving to find a more suitable feeding ground (Mercier *et al.*, 1999a, b).

5.6. Substratum preferences and selectivity

The substratum preferences of juvenile *H. scabra* >10–140 mm have recently been investigated (Mercier *et al.*, 1999a, b). A clear preference was shown for a sand with medium-sized grains of around 0.4 mm. The second choice was a finer muddy sand, while coarse sand and crushed coral were avoided. In all cases, preferences were firmly established within 1 or 2 h, and persisted throughout the experiments, which extended over 24 h.

Although the substrata were washed before testing substratum preferences based on grain size, the residual organic matter contents of the different sediments do not seem to substantiate the hypothesis that some grain sizes are preferred because they provide more organic matter. Medium sand was preferred even though it was not the substratum with the highest proportion of organic matter. However, organic content might not be an important variable at the low values measured in the washed sediments. Alternatively, sea cucumbers may compromise between burrowing energetics and feeding efficiency. Thus medium sand may constitute an ideal substratum because it retains a sufficient load of organic material, and is easy to ingest and to burrow into. Coarser media may be harder to process through the gut, especially for small specimens, while organic content is probably less readily available because of the lower surface/volume ratio. Muddy sand appeared to provide a suitable substratum, but it was largely avoided when opposed to medium sand, possibly because mud does not offer the ideal conditions for burrowing. The fact that juveniles were burrowed deeper in muddy sand might indicate that

this type of substratum has a tendency to draw them in, so that more energy may be required during surfacing (Mercier *et al.*, 1999a).

Although the influence of residual organic content was apparently not the determining factor in the grain size preference, the presence of organic matter was a strong attractant for juveniles foraging on medium sand. Most juveniles wandered to the richest sectors of tanks within an hour, suggesting a strong detection ability (Mercier *et al.*, 1999a). The preference for a richer substratum was firmly established after 2 h and the distribution of juveniles did not vary significantly after 15 h. The organic contents measured at the beginning and at the end of the experiment revealed that the poor substratum remained essentially unchanged with an initial mean organic content of 1.6% and a final mean organic content of 1.4%, while the rich substratum was depleted, according to the respective initial and final mean values of 9.4% and 3.0%. The juveniles thus removed $71.2 \pm 7.4\%$ of the organic matter present in the rich substratum. Four of the tanks filled with poor substratum were enriched by $48.0 \pm 5.1\%$, whereas the other two were depleted by $58.1 \pm 1.6\%$. Control substrata holding no juveniles did not show any variation in organic matter (Mercier *et al.*, 1999a). In this study, small juveniles were slower to react to organic content, which may reflect the smaller volumes of food they require. The amount of organic matter present in poor substrata can apparently support them and movement to a richer surface might not be profitable. Conversely, larger individuals, needing more nutrients, possibly search for organically rich media in order to maximize their foraging (Mercier *et al.*, 1999a).

Baskar (1994) measured the size of the particles present in the gut of adult *H. scabra* collected from the wild and noted a dominance of muddy grains between 0.13 and 0.25 mm. He concluded that the species was a selective feeder, but did not analyse the substratum composition around the animals. The results of Baskar (1994) could therefore represent only preferential choice of substrata, especially since his data roughly correspond to the grain size of the preferred substratum of the juveniles in the study of Mercier *et al.* (1999a, b).

5.7. Population genetics

Genetic analyses performed on *H. scabra* collected from eight populations from north-east Australia, the Torres Strait and the Solomon Islands identified three distinct groups of populations from the north-east coast of Australia, representing samples from the three regions, Hervey Bay, Upstart Bay and Torres Strait (Uthicke and Benzie, in press b). Populations in the last region were closely connected to those from the

Solomon Islands. Restrictions in gene flow were observed. Moreover, a high proportion of the variation in genetic distances along the east coast of Australia was explained by isolation by distance, suggesting a low dispersal that may reduce the recovery of overfished stocks (Uthicke and Benzie, in press b).

6. REPRODUCTIVE CYCLE

6.1. Sexual dimorphism

The sexes are separate in *Holothuria scabra*, but it is not possible to distinguish between them externally, except during spawning when gonopores are extruded and present a distinct morphology (MPEDA, 1989; Battaglene *et al.*, in press). Battaglene (1999a), Battaglene *et al.* (in press), Mercier *et al.* (2000b) and Morgan (1999b) were able to sex *H. scabra* by using a needle and syringe to sample gametes.

6.2. Sex ratio

Conand (1989, 1993) observed that male *H. scabra* from New Caledonia were slightly more abundant than females, representing about 55% of the population. The sex ratio for *H. scabra versicolor* was 57% in favour of males in New Caledonia (Conand, 1989). However, Conand (1993) indicated that these percentages were not significantly different from a 1:1 ratio. In the Solomon Islands, the male to female ratio was close to 1:1 (Mercier *et al.*, 1999c, 2000b).

6.3. Size at sexual maturity

According to Shelley (1981), *H. scabra* in Papua New Guinea reach sexual maturity at ca. 136 mm length in males and at 199 mm in females. Similarly, Lokani (1995a) estimated that *H. scabra* from Papua New Guinea reached sexual maturity at 140 mm but did not mention the sexes. In India, MPEDA (1989) and D. B. James *et al.* (1994a) indicated that individuals reached sexual maturity after ca. 18 months of growth, when females were 210 mm and males 213 mm long. Baskar and James (1995) noted that size at sexual maturity was between 201 and 230 mm, based on spawning capacities. Conand (1989, 1990, 1993, 1994) indicated that the drained weight at first sexual maturity was 140 g for *H. scabra*,

which corresponds to a total weight of 184 g, identical to the value presented by Harriot (1980) for Moreton Bay (Australia). The values are 320 g for the drained weight, 490 g for the total weight and 220 mm length, respectively, for *H. scabra versicolor*. The great variability in the data probably reflects the various methods used to define the size at sexual maturity. Some used formalin-preserved gonads or histological slides to identify the presence of mature gametes and others based their evaluation on spawning abilities or field spawning observations. Individuals can reach sexual maturity well before being involved in spawning events. Future studies should use standardized methods to define size at sexual maturity.

6.4. Gonad morphology

Male and female gonads are composed of numerous thin, filamentous tubules united basally into one tuft attached to the left side of the dorsal mesentery and hanging freely in the coelomic cavity (Conand, 1989, 1993). The tubules are elongated and branched. At the gonad base, the gonoduct, which possesses an inner ciliated epithelium, proceeds into the mesentery and opens to the outside in the mid-dorsal region near the anterior end (D. B. James, 1989b; Conand, 1998b). Several accounts describe gonad appearance. D. B. James (1989b) indicated that ripe female gonads were brown with the oocytes visible as multiple white spots. Similarly, Mary Bai (1980) noted that the female tubules were brown, uniformly thick and long and that the oocytes were visible as small white spots. These descriptions of the female gonad differ considerably from those of P. S. B. R. James and D. B. James (1993) and Conand (1989, 1990, 1993, 1994) who reported that the ripe ovary was translucent. According to Mary Bai (1980), the testes consist of white, long beaded, uniformly thick and long filaments.

Conand (1990, 1993) and Baskar (1994) described the maturity stages in the gonad of *H. scabra*. According to the latter author, immature individuals possess single and short tufts of tubules; at this stage, the sexes are not distinguishable. Maturing individuals have longer gonadal tubules with a yellow tinge, and the germinal cells become visible. Young mature individuals show the presence of some spermatozoa or oocytes in the gonad. The gonadal tubules in adult males are larger, yellow, and branched with round saccules. The adult female gonadal tubules become yellowish red and are branched. At full maturity, males have long, pale yellow, gonadal tubules with two or three ancillary branches filled with numerous spermatozoa. Baskar (1994) also noted that spent males have shortened, less abundant tubules, with only few remaining spermatozoa, while female

gonadal tubules become shorter and wider after spawning but they retain their colour and a few pockets of remaining oocytes.

Maturing gonadal tubules show a clear sexual dimorphism. The fecund ovarian tubules are shorter, wider and heavier than the testes. The mean ripe gonad weighs ca. 24 g in male and 31 g in female (Conand, 1993). The gonadal tubules in males and females are 82 mm and 80 mm and their diameter is 10 mm and 13 mm, respectively (Conand, 1989, 1990, 1993, 1994). The gonadal index (gonad weight/body weight ratio) is also higher in females than in males (Conand, 1993). The gonadal tubules were also described by Shelley (1981), who reported generally shorter lengths but larger diameters. As for *H. scabra versicolor*, male and female gonads weigh 45.9 g and 69.7 g, respectively and the length of gonadal tubules is 137 mm for males and 125 mm for females, which is noticeably different from *H. scabra* (Conand, 1989, 1990, 1993).

According to Mary Bai (1980), the male spermatogonial cells are attached to the germinal epithelium. The spermatogonial cells are larger and more or less spherical in shape with a distinct nucleus in the centre. Just above the spermatogonial cells, nearer the lumen of the tubules, are the primary and secondary spermatocytes, which are much smaller, with deeply staining nuclei. The mature spermatozoa and sperms are found in the centre of the lumen (Mary Bai, 1980).

The female gonoduct consists of a ciliated epithelium, cells of which are regular in size with long cilia directed towards the lumen, a broad connective tissue and externally a layer of coelomic epithelium continuous with the dorsal mesentery (Mary Bai, 1980). The germinal epithelium gives rise to numerous oogonial cells, which are small and embedded in the germinal epithelium. The oogonial cells have prominent lightly staining nuclei with distinct deeply staining nucleoli. Primary, secondary and mature oocytes are present in the lumen. Before spawning, mature oocytes are characterized by the presence of a very large germinal vesicle with a distinct nucleus (Mary Bai, 1980). Ong Che and Gomez (1985) stated that mature *H. scabra* oocytes varied in shape from pyramidal to elongated and club-shaped and that fully grown oocytes possessed a dense, yolk-filled cytoplasm. A large nucleus with an outer basiphilic cup and an inner basophilic core was also noted, resting eccentrically against the nuclear membrane (Ong Che and Gomez, 1985).

As detailed in Table 6, the oocyte diameter seems to be very variable within *H. scabra*'s geographic range. The considerable differences may reflect the various research protocols. Although methods were not always provided, some studies were performed on histological slides, others on preserved gonads and still others on fresh samples. Methods need to be standardized, or correlations have to be established to compare the different values.

Table 6 Fecundity of *Holothuria scabra* over its geographic range.

Location	Oocyte diameter (μm)	Female fecundity ($\times 10^6$ oocytes)	References
Australia	80–125	–	Harriot, 1980
India	180–200	1	D. B. James *et al.*, 1988; P. S. B. R. James and D. B. James, 1993; D. B. James, 1994g
	180–200	1	D. B. James *et al.*, 1989, 1994b; D. B. James, 1994h
	120–165	–	Baskar, 1994
	180–200 (192*)	1	D. B. James *et al.*, 1994a
New Caledonia	190 (210*)	2–9	Conand, 1990
	210		Conand, 1993
Philippines	70–80	–	Ong Che and Gomez, 1985

*Average values.

6.5. Gametogenesis and evidence of spawning periodicity

The reproductive cycle of *H. scabra* has been extensively investigated over its geographic range. According to most studies, the reproductive cycle comprises resting (resorption), growing, maturing, spawning and post-spawning stages (Conand, 1989, 1990, 1993, 1994; Ong Che and Gomez, 1995; Tuwo, 1999; Morgan, 2000a) or immature, mature, gravid and spent gonads (Krishnaswamy and Krishnan, 1967) (Figure 5). Tuwo (1999) observed that mature tubules contained only vitellogenic oocytes in females and only spermatozoa in males, whereas spent tubules were filled with relicts of oocytes or spermatozoa. Conand (1993) indicated that some ovaries in the resorption stage were filled with small oocytes, some large residual oocytes and degenerating cells, while others were empty. The process of resorption could be spread over months (Conand, 1993). However, Ong Che and Gomez (1985) indicated that a rapid recovery to the growing stage occurred after spawning.

The levels of RNA and DNA seem to change distinctly in different states of gonad maturity (Krishnan, 1967). In males, both RNA and DNA become more abundant with maturity. In females, oocyte maturation is associated with increasing RNA and decreasing DNA (Krishnan, 1967). Krishnan (1968, 1970) and Krishnan and Krishnaswamy (1989) observed that accumulation of organic components occurred in the

A
PO
VO
L
E
SZ
B
VO
O
PO
F
SZ
C
O
G
SZ
D
PO
H
RS

gonad when they reached maturity. Proteins and lipids were found to be important storage materials. Intestinal proteins and lipids were utilized by the gonads during the reproductive cycle (Krishnan, 1968, 1970). Conversely, Morgan (1999b) proposed that *H. scabra* was able to use nutrients from gonads and gametes for somatic metabolism during food deprivation.

Studies of gametogenetic cycles have shown that at least a small proportion of *H. scabra* populations seem to spawn all year round (Krishnan, 1968; Ong Che and Gomez, 1985; Shelley, 1985b; Conand, 1989, 1990; Battaglene, 1999b; Tuwo, 1999), although two major spawning peaks were noted in most regions (MPEDA, 1989). Table 7 summarizes the different results. Both Krishnaswamy and Krishnan (1967) and Moiyadeen (1994) indicated that the reproductive cycle was more sharply defined in females than in males. According to Moiyadeen (1994), the gonadal index showed two annual peaks, thus suggesting a bi-annual reproductive cycle in Sri Lanka. His results were confirmed by larval abundance in the field. A similar pattern with two peaks was observed in India (Krishnaswamy and Krishnan, 1967), Australia (Harriot, 1980), Indonesia (Tuwo, 1999) and Papua New Guinea (Shelley, 1981). In New Caledonia, Conand (1989, 1990, 1993, 1994) identified – from morphological and gonadal index variations – a first well-marked peak from December to February, followed by a smaller more variable one between August and October. Cowan and Gomez (1982), Ong Che and Gomez (1985) and Tuwo (1999) found that the gonadal index remained high and that spawners were abundant all year round in the Philippines and Indonesia, respectively, but that two or three major spawning peaks apparently occurred. Similarly, Battaglene (1999b) and Ramofafia *et al.* (1999, in press) found a marked spawning peak between September and October in the Solomon Islands. Morgan (1999b, 2000a) indicated that the gonadal index peaked in November in Australia.

Figure 5 Photomicrographs of histological slides illustrating the different maturity stages of male and female gonads of *Holothuria scabra* in Indonesia. (A) Female early stage with previtellogenic oocytes (PO) and vitellogenic oocytes (VO) growing near the germinal epithelium. The lumen (L) is almost free of gametes. (B) Maturing female gonad with previtellogenic oocytes (PO), vitellogenic oocytes (VO) and mature oocytes (O). (C) Mature female gonad full of mature oocytes (O). (D) Post-spawning female gonadal tubules filled with some residual oocytes (RO). (E) Male gonadal tubules in the early maturing stage showing spermatocytes (SS). (F) Male maturing gonadal tubules filled with layers of spermatocytes (SS) close to the germinal epithelium and spermatozoa (SZ) in the central part of the lumen. (G) Male fully mature gonadal tubules with lumen filled with spermatozoa (SZ). (H) Gonadal tubules after spawning with residual spermatozoa (RS). The horizontal bars represent 50 μm. (From Tuwo (1999) with permission.)

Table 7 Estimated spawning periods of *Holothuria scabra* and respective known spawning cues.

Location	Spawning period	Spawning cue	References
Australia	September–November	–	Morgan, 2000a
	Two peaks	–	Harriot, 1980
Egypt	April–June	–	Mortensen, 1937
India	March–May and November–December	–	MPEDA, 1989
	July and October	Change in salinity and temperature	Krishnaswamy and Krishman, 1967
	March–May and October–December	–	P. S. B. R. James and D. B. James, 1993; D. B. James *et al.*, 1994a; D. B. James, 1996
	March–April and September–October	–	Mary Bai, 1980; D. B. James, 1989b
	July and October, but seem to spawn all year round	–	Bakus, 1973
Indonesia (Sulawesi)	March–July and November–January, but seem to spawn all year round	Temperature variation	Tuwo, 1999
New Caledonia	December–February and August–September	Coolest and warmest temperatures	Conand, 1990, 1993, 1994
Papua New Guinea	Austral Summer	–	Shelley, 1985b
	November–January	–	Lokani, 1995a
	December–February	–	Lokani *et al.*, 1995b
Philippines	May–June and November–January, but seem to spawn all year round	When temperature and salinity change drastically	Ong Che and Gomez, 1985
	Three gonadal index peaks	–	Cowan and Gomez, 1982
Red Sea	June–August	–	Boolootian, 1966
Solomon Islands	May and November	–	Battaglene *et al.*, 1998
	September, but spawning was evident all year round	–	Ramofafia *et al.*, 1999; Mercier *et al.*, 2000b
Sri Lanka	April–November (male) and March–October (female)	Salinity changes due to rainfall	Moiyadeen, 1994

The dominant patterns in variation of the reproductive cycle between different geographical areas are represented well by the studies done in India, New Caledonia, Indonesia and the Solomon Islands. Krishnaswamy and Krishnan (1967) studied the reproductive cycle of *H. scabra* in India using gonadal indices and histological examination of fresh and preserved gonads. The gonadal index increased drastically between June and July, followed by a sharp drop in August. In September, a second important increase was noted and a low value was recorded in November. Although mature animals were found almost throughout the year, greater proportions were identified before both gonadal index peaks.

Conand (1989, 1990, 1993), who also studied gonadal indices and the different maturity stages of the gonads, indicated that *H. scabra* were in the resting period around June, while the growing period overlapped July and August, in New Caledonia. A spawning event occurred between August and September followed by another growing stage in mid-September and October. The maturing stage in November was followed by a long spawning period between the end of November and February, followed again by a period of growth between February and May. Female *H. scabra* apparently do not release all mature oocytes during a spawning event, hence the resting period is short. This supports the hypothesis that *H. scabra* can spawn throughout the year. *H. scabra versicolor* from New Caledonia presents a more distinct reproductive cycle with only one annual spawning period (Conand, 1989, 1990, 1993). After a resting stage from mid-April to May, the gonadal tubules enter a growing stage until the end of September. A short maturing stage in October is followed by a spawning period between November and February and a post-spawning period between February and April.

Tuwo (1999) used similar techniques to those used by Conand (above) and found that the reproductive cycle of *H. scabra* in Indonesia showed a maturation period extending from July to March. Post-spawning stages were noted mainly at the beginning of the dry season, from March to July, and during the beginning of the rainy season, from November to January. Gonad growth occurred in two phases, first from June to October and then from February to April. A proportion of individuals in the maturation stage possessed mature tubules, so that partial spawning events could occur at any time of the year (Tuwo, 1999). Similarly, Ong Che and Gomez (1985) proposed that staggered gametogenesis among different individuals permitted continuous breeding for the population as a whole in the Philippines. However, they observed that gonad maturation occurred from January to April and from July to October, with main spawning events occurring from May to June and from October to November.

Morgan (2000a) indicated that there was a continuous presence of mature oocytes that were either reabsorbed or spawned during or before the vitellogenic period from September to November in Australia. Morgan (2000a) further mentioned that it was likely that stored nutrients in the body wall were used for gametogenesis during the latter part of winter and that oocyte production was regulated by phagocytic activity.

Ong Che and Gomez (1985) also studied gametogenesis using seasonal fluctuations of oocyte diameter and thickness of the different layers of maturing male gametes. Both oogenesis and spermatogenesis appeared to be continuous. Analysis of the size-frequency structure of maturing oocytes showed that oocytic growth was not perfectly synchronized among females of each sample. While two or three cohorts of oocytes could be observed, unimodal distribution was noted most of the time. The size distribution of oocytes did not show any seasonal variation, with a constant peak mode around 70–80 μm in diameter. Interestingly, Battaglene (1999a) and Battaglene *et al.* (in press) suggested that gonadal maturity of *H. scabra* could not be accurately gauged from oocyte diameters, but needed to be confirmed by a 5–8% change in the gonadal index. Ong Che and Gomez (1985) noted that the mean thickness of the spermatozoa and spermatogenic cells layers were inversely related during the reproductive cycle. As the gonad ripened and matured, spermatozoa were recruited from the spermatogenic cell pool and the spermatogenic cell layer thinned out while the spermatozoa layer increased in thickness. During spawning and subsequent gamete growth and multiplication, the spermatozoa layer became thinner while the mean thickness of the spermatogenic cell layer increased.

Investigations of the reproductive cycle in *H. scabra* have been done using similar but non-uniform techniques. The use of the drained weight for the calculation of indices in sea cucumbers has long been a matter of debate. Some researchers remove the intestine, whereas others remove only the water content in the Polian vesicle and in the respiratory tree. Obviously, these differences introduce important variability. The most accurate method is to remove all internal organs that could induce variability and weigh only the well-blotted body wall with the aquapharyngeal bulb (Conand, 1989, 1990). The dry weight of body wall would be even more reliable, but difficult to use for this large species.

6.6. Influence of environmental factors on gametogenesis

In the Philippines, gamete synthesis increased with temperature during summer and decreased at the end of the year when the temperature dropped (Ong Che and Gomez, 1985). Krishnaswamy and Krishnan

(1967) suggested that salinity variations could best explain the yearly reproductive cycle of *H. scabra* in India. However, Conand (1989, 1993, 1994) found no clear correlation between salinity and the reproductive cycle in New Caledonia, but did observe a temperature effect during the warmest and coolest periods. Battaglene (1999a, 2000) and Battaglene *et al.* (in press) noted that the peak of the reproductive season (October) was towards the end of the dry season in the Solomon Islands, suggesting that both salinity and temperature fluctuations could provide the proximal cues that synchronize and regulate the seasonal reproductive cycle. Morgan (2000a) suggested that the onset of gamete maturation may be correlated with a change in photoperiod and that further development could be associated with the food availability and temperature. Finally, Mercier *et al.* (1999c, 2000b, in press a) found that the lunar phase could play a role in the pairing behaviour and gametogenic synchrony among *H. scabra* populations in the Solomon Islands.

6.7. Fecundity

Harriot (1980) indicated that there was no relationship between the fecundity of *H. scabra* and its body size. Variability in fecundity data provided for *H. scabra* (Table 6) can probably be attributed to methodology artifacts. For instance, P. S. B. R. James and D. B. James (1993) measured fecundity using the number of spawned oocytes only, thus presenting values of ca. 1 million oocytes per female, whereas Conand (1989, 1990, 1993, 1994) evaluated potential fecundity by dissecting mature gonads and proposed much higher values of >2–18 million oocytes per female, with the higher values for the larger females. As *H. scabra* has been suggested to participate in partial spawning events, and as only part of the mature gametes are shed during artificially induced spawning (S. C. Battaglene, unpublished data), the only reliable fecundity data would be those obtained using the whole gonads. Conand (1989, 1993) found that the absolute fecundity of *H. scabra versicolor* varied between 9 and 17×10^6 oocytes per female and was correlated with body size.

6.8. Asexual reproduction

Lokani *et al.* (1995b) induced fission in *H. scabra* by constriction of the body wall for a week. They observed that the two resulting sections regenerated to form fully developed sea cucumbers. However, to our knowledge, fission has never been reported to occur naturally in this species.

7. SPAWNING

7.1. Behaviour

The spawning behaviour of *Holothuria scabra* is described quite uniformly in the literature, although very few details are given to differentiate the sexes (MPEDA, 1989). During spawning individuals lift the anterior part of their body and initiate a sweeping movement. At the same time, the genital papilla, which is located anteriorly on the dorsal surface, dilates (Conand, 1993). The anterior region of the female's body inflates due to accumulation of gametes in the gonoduct (P. S. B. R. James and D. B. James, 1993; D. B. James *et al.*, 1994a). D. B. James *et al.* (1994b) add that the male aquapharyngeal bulb is fully extended during gamete release. During spawning, sperm or oocytes are released through the gonopore by the ciliary action of the gonoduct (Mary Bai, 1980; D. B. James, 1989b). The sperm or oocytes are discharged as a continuous stream, a white cloud that is not always easily visible (Conand, 1989, 1993). However, some reports indicate that gamete broadcasting differs between males and females. For example, males have been observed to release sperm continuously, whereas females release oocytes by powerful intermittent jets or spurts over about 2–3 h (MEPDA, 1989; P. S. B. R. James and D. B. James, 1993; D. B. James *et al.*, 1994a, b; Battaglene, 1999a, 2000; Battaglene *et al.*, in press). P. S. B. R. James (1996) indicated that females expelled their gametes in one or two spurts. D. B. James (1994h) gave sperm release duration of 30–60 min, whereas MPEDA (1989) observed that male spawning lasted about 2 h. D. B. James *et al.* (1988) mentioned that males spawned during 15–20 min and Lokani (1995a) indicated that females released oocytes in cycles of about 5 min. Spawning behaviour has also been observed in *H. scabra versicolor* and is illustrated by Conand (1989, 1993).

The released oocytes are spherical, buoyant, white and visible to the naked eye (D. B. James *et al.*, 1988; Anonymous, 1989; P. S. B. R. James and D. B. James, 1993), although D. B. James *et al.* (1994a) also noted that oocytes are light yellow when spawned.

7.2. Influence of environmental factors and timing

Salinity variation has been proposed as the major factor triggering gamete shedding in India (Krishnaswamy and Krishnan, 1967). However, this does not seem to be the case in New Caledonia (Conand, 1990, 1993, 1994), the Philippines (Ong Che and Gomez, 1985), or Indonesia (Tuwo, 1999),

where temperature variation is the most probable spawning cue (Table 7). Moiyadeen (1994) found that a mix of salinity and temperature variations as well as food availability could trigger spawning of *H. scabra* in Sri Lanka. P. S. B. R. James and D. B. James (1993), D.B. James *et al.* (1994a, b) and MPEDA (1989) observed that males released their gametes first, around noon, followed by females minutes or hours later. This raises the possibility that male sperm can induce nearby females to spawn, but is not strong evidence that sperm are the cue. D. B. James (1989b) and Lokani (1995a) also observed that male *H. scabra* spawned first. However, Krishnaswamy and Krishnan (1967) noted that male and female *H. scabra* spawned simultaneously.

Shelley (1981) found that spawning of *H. scabra* in the wild occurred between 1000 h and 1600 h during the new moon. Observations in holding tanks with or without stimulation roughly concur. D. B. James (1994h) indicated that males usually spawned around 1000 h and females around 1400 h. Similarly, MPEDA (1989) mentioned that eggs were mostly released at around 1500 h, whereas Battaglene (1999a, 2000) and Battaglene *et al.* (in press) reported that spontaneous spawners released their gametes between 1500 h and 1800 h, with males spawning first.

Morgan (2000b, in press a) reported that spawning induction was most effective at dusk around new or full moons, and Battaglene (1999c) observed that 33% of *H. scabra* spawned 2 d after the new moon in outdoor tanks. Mercier *et al.* (1999d, 2000a, in press b) noted that newly settled pentactulae are present on seagrass leaves before the full moon, suggesting that spawning and fertilization take place around the full moon. Conand (1993) noted that *H. scabra versicolor* has been observed spawning 2 d after the first moon quarter.

Although numerous studies have concluded that spawning occurs all year round, cues for synchronous spawning of males and females, which would optimize fertilization, have rarely been investigated. One indication of synchronous spawning is aggregative behaviour prior to spawning. Such behaviour occurs in the Solomon Islands, where it is correlated with the lunar cycle (Hamel *et al.*, 1999; Mercier *et al.*, 2000b). The aggregative behaviour of *H. scabra* suggests that chemical communication may be occurring. In the observations from the Solomon Islands, most individuals remained away from each other in the absence of a moon, and started to form pairs, trios and larger groups progressively after the new moon. Aggregation peaked a few days before the full moon when >95% of the individuals participated, and subsequently decreased until the next new moon. Most of the time, pair formations were more common than larger aggregations, with no progressive pattern or correlation to sex. Male and female spawnings were observed in outdoor tanks, typically around the full moon. Spawning occurred both during the peak in aggregation and later

on, when the individuals were more evenly distributed (Mercier *et al.*, 2000b). No aggregation was noted in individuals <110 mm in length. Conversely, Van den Spiegel *et al.* (1992) mentioned that they never observed any aggregative behaviour in *H. scabra* populations from Papua New Guinea.

7.3. Artificial induction of spawning

D. B. James (1994h, undated) and P. S. B. R. James (1996) observed that individuals collected during the breeding season released their gametes without any external stimulus other than the stress of collection. Nonetheless, thermal shock is the most widespread technique used to induce *H. scabra* to spawn. Typically, mature individuals are transferred to water that is ca. 3 or 5°C warmer than that in the original holding tank, and maintained at this temperature for several hours, if necessary (D. B. James *et al.*, 1988, 1989, 1994b; MPEDA, 1989; P. S. B. R. James and D. B. James, 1993; D. B. James, 1994a, g; P. S. B. R. James, 1996; Battaglene *et al.*, 1998, in press; Battaglene, 1999a, 2000; Morgan, 1999b, 2000b, in press a). Although results seem to vary with the protocol of thermal stimulation, the seasonal gonadal maturity and lunar periodicity, the most reliable period for inducing spawning artificially was September in the Solomon Islands (Battaglene, 1999a, 2000; Battaglene *et al.*, in press) and October–January in Australia (Morgan, 2000b). Battaglene (1999a, 2000) and Battaglene *et al.* (in press) noted that it was easier to induce spawning in males. It has been reported that thermally-shocked females could be induced to spawn even in the absence of sperm, but that males usually spawned first, suggesting that thermal stress might trigger male spawning and that females are stimulated by the presence of sperm in the water column (D. B. James *et al.*, 1994a). Recently, Ramofafia *et al.* (1999, in press) and Battaglene (1999b) were able to trigger spawning in mature females by adding a solution of dried algae (*Schizochytrium* sp.) to the holding tank. The use of a powerful jet of water on drying individuals also increased the incidence of spawning (P. S. B. R. James and D. B. James, 1993; D. B. James, 1994h, 1996, undated), whereas stripping gonads was effective only to a certain degree (P. S. B. R. James and D. B. James, 1993; D. B. James, 1994h, undated; P. S. B. R. James, 1996). Mercier and Hamel (unpublished data) and Battaglene (unpublished data) had no success when gametes collected by stripping gonads were used in fertilization trials. Final maturation of the oocyte was reported to take place during spawning and fertilization (Ong Che and Gomez, 1985).

8. DEVELOPMENT

General trends in the development of *Holothuria scabra* are relatively uniform throughout the existing literature (Table 8), although many strange reports and discrepancies appear in the fine details. Development starts immediately after the release of oocytes, fertilization occurs in the water column and larval development is planktotrophic with feeding auricularia larvae transforming into non-feeding doliolariae before settling as pentactulae (Figure 6). Figure 7 schematically illustrates the life cycle of *H. scabra* from spawning to post-settlement.

A number of studies pertaining to the development of *H. scabra* have been conducted by Indian researchers. P. S. B. R. James (1996) observed that spawned oocytes sank to the bottom, while D. B. James *et al.* (1988) stated that development took place at the surface of the water column. P. S. B. R. James and D. B. James (1993) noted that first cleavage occurred 15 min after fertilization and that the first polar body appeared after 20–30 min, although we find this sequence very unlikely. The gastrula (dipleurula) measured 190–256 μm (D. B. James *et al.*, 1988) and was described as a mobile stage (P. S. B. R. James and D. B. James, 1993). After 48 h, the larvae reach the auricularia stage and they are fully formed after 5 or 6 days (D. B. James, 1994h, undated; D. B. James *et al.,* 1994a; P. S. B. R. James, 1996). The auricularia is a slipper-shaped, transparent and pelagic larva that possesses a preoral loop anteriorly and an anal loop posteriorly; there are a number of pigment spots on the anal section (P. S. B. R. James and D. B. James, 1993; D. B. James, 1994h, undated; P. S. B. R. James, 1996). At this stage, the digestive tract consists of a

Table 8 Embryonic development of *Holothuria scabra.**

Stage of development	Time
First cleavage	15 min
Four-cell stage	20 min
Early blastula	40 min
Fully formed blastula	3 h
Gastrula	24 h
Early auricularia	48 h
Late auricularia	5–6 d
Doliolaria	10 d
Pentactula	13 d
8 mm long juveniles	1 yr

*According to D. B. James *et al.* (1988, 1989, 1994a, b), MPEDA (1989), P. S. B. R. James and D. B. James (1993), D. B. James (1994g), Manikandan (2000).

Figure 6 Larval stages of *Holothuria scabra*. (A) auricularia (ca. 560 μm), (B) doliolaria (ca. 460 μm), and (C) pentactula (ca. 600–700 μm). (Photographs courtesy of S. C. Battaglene, ICLARM.)

Figure 7 Schematic life cycle of *Holothuria scabra*. (From Battaglene (1999a) with permission; drawing by S. Pallay, ICLARM.)

mouth, an elongated pharynx and sacciform stomach, enabling the larvae to feed on planktonic matter (D. B. James *et al.*, 1988). Auricularia larvae measure about 563 μm at the early stage and 1.1 mm in length when fully developed (P. S. B. R. James and D. B. James, 1993; James, 1994h, undated; P. S. B. R. James, 1996). They are 240–690 μm wide (D. B. James *et al.*, 1988).

Continuing with data from India, the doliolaria has been described as a barrel-shaped larva with five bands around the body and two projecting tentacles (P. S. B. R. James and D. B. James, 1993; D. B. James, 1994h; P. S. B. R. James, 1996). At this stage, larvae are still pelagic and measure between 460 and 620 μm in length (D. B. James *et al.*, 1988, 1994a; P. S. B. R. James and D. B. James, 1993; D. B. James, 1994h; P. S. B. R. James, 1996) and 240–390 μm in width (D. B. James *et al.*, 1988). Larvae are generally said to metamorphose into pentactulae after 13 d, although P. S. B. R. James (1996) indicated that metamorphosis started between the auricularia and doliolaria stages, 10 d after fertilization. The pentactula is described as tubular with five buccal tentacles and a single stumpy tube foot at the posterior end (P. S. B. R. James and D. B. James, 1993; D. B. James, 1994h; P. S. B. R. James, 1996). The cloacal opening is distinct, and the colour of the body is greenish brown. At this stage, the young sea

cucumber becomes benthic. The pentactula is about 600–700 μm long (P. S. B. R. James and D. B. James, 1993; D. B. James *et al.*, 1994a) and 250–400 m wide (D. B. James *et al.*, 1988). Finally, 18 days after fertilization, the tube feet and tentacles become more distinct, including two large tube feet at the posterior end. The spires of the tables project from the body wall (D. B. James *et al.*, 1988).

Studies in the Solomon Islands are consistent with the above reports from India, but the time frame differs. In particular, the blastula forms within 40 min and the whole planktonic cycle lasts 10–14 days at 27°C (Battaglene, 1999a, 2000; Battaglene and Bell, 1999; Battaglene *et al.*, 1999, in press). The pentactula forms 13 days after fertilization and marks the transition from planktotrophic to benthic life style (Ramofafia *et al.*, 1999, in press), as corroborated by most studies (MPEDA, 1989) (Table 8). Battaglene (1999a, 2000) and Battaglene *et al.* (in press) indicated that the presence of distinct hyaline or lipid spheres at the late auricularia stage appeared to be a good indication of larval competency for metamorphosis.

9. SETTLEMENT

Settlement and post-settlement processes of *Holothuria scabra* have been studied in the laboratory (Mercier *et al.*, 1999d, 2000a, in press b). Independent and paired choice experiments revealed that several substrata could induce metamorphosis of doliolaria into pentactula, but that specific substrata favoured settlement. Leaves of the seagrass, *Thalassia hemprichii*, with or without their natural biofilm, yielded the highest settlement rates (4.8–10.5%). *T. hemprichii* was preferred as a settlement substratum over sand, crushed coral, several other plant species and artificial seagrass leaves with or without a biofilm. Only settlement on another seagrass, *Enhalus acoroides*, was similar to that recorded for *T. hemprichii* (Mercier *et al.*, 2000a). In the absence of a suitable substratum, the larvae delayed settlement for nearly 96 h and survival was <0.5%. Sand and crushed coral, either alone or together, attracted settlement from <1.5% of the available larvae. The pentactulae found on sand, coral and in bare containers were 10–35% smaller than those on *T. hemprichii* leaves. Introduction of soluble extracts from *T. hemprichii* and *E. acoroides* successfully induced metamorphosis and settlement onto clean plastic surfaces.

Field studies in the Solomon Islands by Mercier *et al.* (1999c, 2000b, in press a) showed that several *H. scabra* <10 mm were found on leaves of *Enhalus acoroides* and *T. hemprichii* but not on other substrata. The

highest density of newly settled and early juveniles of *H. scabra*, corresponding to the smallest average size of 1.7 mm, were found typically before the full moon. In contrast, the lowest densities, comprising individuals of 5.4 mm, were usually observed 12–16 days after the full moon. This pattern was repeated each month, except between September and November in 1998. Also, samples from December 1998 and January and February 1999 revealed very low or zero recruitment (Mercier *et al.*, 2000b). The occurrence of other fauna on the seagrass, including numerous potential predators such as polychaete worms, tectibranchs, crabs, gastropods, copepods, shrimps, flatworms and isopods, varied monthly in number and diversity. Maximum abundance of potential predators was correlated with peak recruitment of young *H. scabra* and could explain their rapid decrease in abundance (Mercier *et al.*, 2000b).

Most studies indicate that settlement occurs at the pentactula stage (MPEDA, 1989), but some research teams whose work focused on aquaculture techniques (e.g. P. S. B. R. James and D. B. James, 1993; D. B. James *et al.*, 1994a), indicated that *H. scabra* settled at the doliolaria stage. In the latter studies, settlement was stimulated by adding extracts of finely filtered *Sargassum* to the tank so that larvae settled on polythene sheets. P. S. B. R. James (1996) also indicated that pentactulae settled on hard substrata with a diatom and bacterial film in the laboratory. More recently, Battaglene (1999a, 2000), Battaglene and Bell (1999) and Battaglene *et al.* (in press) found that *H. scabra* larvae settled onto plates conditioned with diatoms and biological films. Battaglene (1998a, 1999b) noted that *H. scabra* settled on conditioned plates about 2 weeks after fertilization.

10. MIGRATION

Vail (1989) and Uthicke and Benzie (1998) found that *Holothuria scabra* from a seagrass bed <2 m depth were smaller than individuals found in deeper water along the coast of Australia, supporting the idea that seagrass beds are nursery habitats for this species. Uthicke and Benzie (1998) also observed that a greater proportion of adult *H. scabra* are sexually mature in deeper water. Mercier *et al.* (1999c, d, 2000a, b, in press a, b) recently observed the settlement of pentactula larvae on seagrasses *Thalassia* and *Enhalus* in the Solomon Islands, both in the laboratory and in the field. Newly settled juveniles remained on the seagrass for 4–5 weeks before migrating to sand at around 6 mm in length. Before this, the juveniles spent 4–5 days moving on and off the seagrass. Once on the sand, the juveniles did not show the typical burrowing behaviour of older specimens

until they reached around 11 mm in length. The larvae of *H. scabra* appear actively to select seagrass, possibly through chemical detection. Mercier *et al.* (2000a, b) hypothesized that larvae settling on seagrass have an increased chance of growth and survival because they are provided with a suitable substratum on which to grow, and a bridge to sandy substrata.

In the field, juveniles remain on sand among seagrasses until they reach ca. 90–100 mm and then move to the open sandy area or mud flat. Larger individuals are localized in deeper water >1.5 m (Mercier *et al.*, 2000b). D. B. James *et al.* (1994a) also noted that in India *H. scabra* migrated to deeper water for breeding. A greater number of large and sexually mature individuals were observed in deep areas by Uthicke and Benzie (1998, 1999) in Australia, supporting the hypothesis that there is a downward migration of growing individuals. However, Harriot (1980) and Mercier *et al.* (2000b) observed mature individuals in shallow waters in Australia and the Solomon Islands, respectively, suggesting that spawning also occurs there. Uthicke and Benzie (1999) determined that the deep and shallow populations are genetically linked and demonstrated that both groups of individuals are derived from the same larval pool. In Madagascar, Conand (1997b) observed a dense bed of juveniles on an intertidal flat, near to a 20 m deep muddy bay where a prawn fishery trawls large individuals as a by-catch. Interestingly, at Ilot Maitre in New Caledonia, Conand (1989) found mature *H. scabra* mixed with *H. scabra versicolor* on the inner reef flat covered by a dense seagrass bed, while at the bottom of the slope (20 m), *H. scabra versicolor* was mixed with *Stichopus hermani* without any *H. scabra*.

Another type of migration was noted in Mozambique after a massive organ evisceration: individuals initially found among seagrasses and algae close to the littoral moved to deeper water with the outgoing tide (Anonymous, 1996c).

A tagging or chemical marking method adapted for long-term studies is still needed to confirm the downward migration hypothesis.

11. GROWTH

Numerous references to the growth rate of *Holothuria scabra* adults and juveniles, recorded under laboratory conditions, can be found in the literature. Average values range from 0.07 to 1.5 cm mo^{-1} in rearing tanks, depending on initial size and growth period (Table 9). Corresponding weight gain has been estimated by a few authors to be between 0.2 and 0.9 g d^{-1} (Muliani 1993; Battaglene 1999a, b, c; Battaglene *et al.*, in press) or about 14 g mo^{-1} (Shelley, 1985a, b). When

Table 9 Growth rate of *Holothuria scabra* according to different studies.

Conditions	Initial size	Average growth rate	Period	References
Laboratory	–	1.4 cm mo^{-1}	7 mo	Anonymous, 1978b
Laboratory	100–250 mm	0.5 cm mo^{-1} 14 g mo^{-1}	–	Shelley, 1985a, b
Laboratory	–	1.0 cm mo^{-1}	3 mo	Anonymous, 1989
Laboratory	–	1.13 cm mo^{-1}	1 yr	MPEDA, 1989; D. B. James *et al.*, 1994a
	136 mm	0.74 cm mo^{-1}	1 yr	
	225 mm	0.49 cm mo^{-1}	1 yr	
	284 mm	0.32 cm mo^{-1}	1 yr	
	322 mm	0.22 cm mo^{-1}	1 yr	
Laboratory without food	18 g	0.4 g d^{-1}	–	Muliani, 1993
Laboratory with food	18 g	0.9 g d^{-1}	–	
Laboratory	–	0.07 cm mo^{-1}	1 yr	D. B. James, 1994g
Laboratory	Pentactula	0.1 cm mo^{-1}	30 d	D. B. James *et al.*, 1994a
Laboratory	–	0.7 cm mo^{-1}	1 mo	D. B. James *et al.*, 1994b
Fibreglass and concrete outdoor tanks	10 mm	0.5 mm d^{-1} 0.2 g d^{-1}	1–12 mo	Battaglene, 1999a, b, c, 2000; Battaglene and Bell, 1999; Battaglene *et al.*, 1999, in press
Field	65 mm	10–15 cm mo^{-1}	2 mo	Mercier *et al.*, 2000b
Field	15 mm	1.4 cm mo^{-1}	6 mo	Manikandan, 2000

H. scabra were stocked at a biomass >225 g m^{-2}, growth ceased and some individuals even lost weight (Battaglene *et al.*, 1999). Such lack of growth has also been observed by Conand (1983) and Ramofafia *et al.* (1999).

In contrast, studies of growth in the field are scarce. Mercier *et al.* (2000b) periodically measured hatchery-reared juveniles released in the wild and found that growth rates over 2 mo were dependent upon substratum type: the length of the individuals, which initially averaged 6.5 cm, increased by 385% on sand, by 327% in seagrass and by 252% on shells and crushed coral. This roughly corresponds to a growth rate of 10–15 cm mo^{-1} (Mercier *et al.*, 2000b), which is 10–30 times the usual growth rate found in captivity (Table 9). Manikandan (2000) reported that hatchery-reared *H. scabra* juveniles of 1.5 cm reached 10 cm after 6 months spent in an enclosed lagoon.

According to Long and Skewes (1997) *H. scabra* ca. 18 cm long are ca. 2 yr old and D.B. James *et al.* (1994a) and MPEDA (1989) reported that *H. scabra* could live ca. 10 yr. However, more studies are needed to establish a growth curve in the field over the entire life cycle of the species.

12. DAILY BURROWING CYCLE

12.1. Adults

Holothuria scabra generally move in a sluggish manner and often remain partly or totally buried in the sediment. Battaglene *et al.* (1999), Mercier *et al.* (1999a, 2000b) showed that *H. scabra* in the Solomon Islands spent about half the day burrowed and the other half on the surface. The burrowing of adult *H. scabra* in Palau extended from early in the morning until ca. 1500 h, and individuals remained on the surface at night (Yamanouchi, 1939, 1956). A similar pattern was observed in India (Anonymous, 1978b). Yamanouchi (1939) explained this behaviour as a way to minimize daylight predation, although *H. scabra* is known to be toxic (Halstead, 1965; Bakus, 1968), and Rao *et al.* (1985a, b) and Bakus (1968) stated that predation on adult *H. scabra* was low. Mercier *et al.* (1999a) found that burrowed *H. scabra* did not show any detectable movement, and burrowing/emerging processes took about 30 min.

The burrowing cycle of *H. scabra* in the Solomon Islands varied according to environmental conditions (Mercier *et al.*, 2000b) (Figure 8): on a clear day, individuals <80 mm burrowed at sunrise and surfaced at sunset. Individuals >80 mm presented almost the same, but slightly dephased burrowing pattern, with individuals beginning to surface earlier, in the middle of the afternoon. However, when it rained, 97% of

Figure 8 Daily burrowing cycle of two size classes of *Holothuria scabra* during rainy episodes and under clear weather conditions in the field. Data were recorded in October 1997 and February 1998 (mean ± SE, $n = 2$). (From Mercier *et al.*, 2000b.)

individuals of both size classes remained burrowed all day (Figure 8). On two occasions, the water temperature was found to increase to more than ca. 30°C (with a peak of ca. 37°C) and most individuals were found on the surface, remaining there until the temperature dropped below ca. 28–29°C, and they did not follow their usual burrowing cycle.

Yamanouchi (1956) observed the respiratory rate by counting the movements of the anus during different daily activities in *H. scabra*, and noted an increase when the animals were burrowed, suggesting that higher respiration rates might compensate for reduced diffusion of gases directly through the body wall. The frequency of inspiration between each spouting varied between 1 and 16 when the animals were on the surface, and it was around 3 in burrowed animals (Yamanouchi, 1956). Anal movements continued despite evisceration of all internal organs and loss of body fluids (Yamanouchi, 1939, 1956).

According to Yamanouchi (1939), the burrowing behaviour of *H. scabra* could be under neural control or under an internal clock. Yamanouchi (1956) determined the sensitivity of the anal region to light with a simple experiment and suggested that it played a role in the burrowing cycle. Mercier *et al.* (1999a, b), in a similar experiment, modified the light pattern around juvenile *H. scabra* and successfully triggered a new burrowing cycle.

12.2. Juveniles

Mercier *et al.* (1999a, b) and Battaglene *et al.* (1999) showed that the burrowing cycle of the smallest juveniles (>10–40 mm) was linked to the light regime. These juveniles begin to burrow around sunrise and emerge close to sunset, and their burrowing behaviour is inhibited in continuous darkness (Mercier *et al.*, 1999a, b). Individuals between 40 and 140 mm responded to changes in temperature, burrowing earlier at around 0300 h as temperature declined, and emerging at mid-day. Constant high temperature prevented their burrowing (Mercier *et al.*, 1999a, b). Burrowing and surfacing takes between 5 and 30 min.

A change in burrowing behaviour was observed when *H. scabra* between 10 and 140 mm were exposed to decreasing salinity conditions (Mercier *et al.*, 1999a). In fact, when exposed to salinities of 25 p.s.u. and lower, juvenile *H. scabra* burrowed rapidly in the sediment even when they would normally be at the surface. Acclimation was observed at salinities of 30, 25 and 20 p.s.u., whereas lower values were beyond their tolerance threshold (Mercier *et al.*, 1999a). This burrowing behaviour was also observed in the field during the rainy season in the Solomon Islands (Mercier *et al.*, 2000b) (Figure 8). The intestinal transit time and intestinal index both decreased

significantly in all size classes of juveniles during salinity decreases (Mercier *et al.*, 1999a). Several burrowed juveniles were observed to forcibly eject faecal pellets within the first hour. The intestinal index decreased significantly within 30 min in small and medium juveniles but only after 10.5 h in large juveniles. The burrowing of juvenile *H. scabra* at low salinity may help them to better equilibrate ionic concentrations of coelomic fluid. Yamanouchi (1956) found that burrowed *H. scabra* exhibited fewer inhalations compared with wandering animals, suggesting that burrowing might reduce the entry of hypohaline water inside the respiratory tree.

Lowering of water level in the laboratory and in the intertidal region also influences the burrowing behaviour (Mercier *et al.*, 1999a, 2000b). Specifically, a steadily decreasing level does not prevent the juveniles from emerging, but a constant low water level significantly reduces emergence. This suggests that the juveniles need a few hours to determine whether the water level has become inappropriately low. However, any such mechanism does not apply to all individuals, as nearly 40% of small juveniles were found on the surface during their "normal" emergence period, even though they were barely covered with water (Mercier *et al.*, 1999a). Similarly, D. B. James (1994b) once reported that individuals living buried inside the sand came out during low tide to lie in a half-burrowed condition.

The response of *H. scabra* to low water levels, combined with their ability to tolerate and acclimate to low salinity, may reflect their adaptation to shallow, tidal habitats. Data seem to demonstrate that smaller juveniles can better tolerate variable conditions than larger animals. The advantages of such adaptability may include reduced competition and predator avoidance.

13. FEEDING BEHAVIOUR, DIET AND SELECTIVITY

13.1. Larvae

The planktonic larvae of *Holothuria scabra* need to feed on pelagic microalgae to complete their development, although feeding is limited to the auricularia stage (Battaglene *et al.*, 1999). The feeding mechanism of the auricularia larvae consists of conveying unicellular algae and suspended fragments of organic matter into the alimentary canal by ciliary movement (D. B. James, 1994h). The same observations were also made by D. B. James *et al.* (1988, 1989, 1994a, b), Anonymous (1989), MPEDA (1989), P. S. B. R. James and D. B. James (1993) and P. S. B. R. James

(1996). Food accepted initially by larvae was <40 μm, but up to 80 μm after 1 mo of growth (P. S. B. R. James and D. B. James, 1993; D. B. James *et al.*, 1994a).

Makatutu *et al.* (1993) tested different species of algae for the growth rate and survival of *H. scabra* larvae and indicated that they grew more rapidly when fed with *Chaetoceros ceratosporum*. Morgan (1999b, 2001b) indicated that larvae provided with excess amounts of algae *Isochrysis galbana* (40–80 cells ml^{-1}) had lower survival and growth rates compared with larvae fed with algal concentrations of between 10 and 20 cells ml^{-1}. When individuals were fed with different larvae, growth declined as follows: *C. cerastosporum* > *Tetraselmis chuii* > *Nannochloropsis oculata* (Morgan, 1999b, 2001). In similar studies by Battaglene (1999a, b, 2000) and Battaglene *et al.* (in press), growth declined as follows: *Rhodomonas salina* > *Chaetoceros muelleri* > *C. calcitrans*.

13.2. Juveniles and adults

H. scabra are generally categorized as deposit feeders and diet descriptions are relatively uniform in the literature. When on soft bottoms, they are said to ingest large amounts of sediment from which they extract food (Baskar, 1994; Conand, 1994, 1999a). To do so, they use retractile tentacles, devoid of calcareous deposits, that can be withdrawn into the mouth (Mary Bai, 1980). *H. scabra* are also described as detritus feeders (Morgan, 1996). Gut contents are generally composed of bacteria, copepods, diatoms and other algae, molluscan shells, foraminiferans, sand and mud (Bakus, 1973; Mary Bai, 1980; Massin, 1982; Sipahutar and Soeharmoko, 1989; Wiedemeyer, 1992; Baskar, 1994; P. S. B. R. James, 1996). D. B. James (1989b, 1996) suggested that *H. scabra* had no food preference.

When moving on compact substrata *H. scabra* are thought to feed only on deposited bacteria and algae on the surface without ingesting sediment (P. S. B. R. James, 1996). In the Solomon Islands, Battaglene and Bell (1999) observed that cultured juveniles fed on epiphytic algae and bacteria growing on the substratum. Battaglene *et al.* (1999) later observed that they fed on sand but also from the hard surfaces of the tanks.

Descriptions of the feeding cycle of *H. scabra* are comparatively disparate. Yamanouchi (1956) suggested that *H. scabra* fed for at least one-third of the day by collecting food with their buccal tentacles while moving on the substratum. D. B. James (1989b) and P. S. B. R. James (1996) specified that *H. scabra* were continuous feeders, and P. S. B. R. James (1996) indicated that the peltate tentacles shovelled the sand and

mud continuously into the mouth. However, Wiedemeyer (1992) reported that they stopped feeding when they were on the surface. Recently, Mercier *et al.* (1999a, b) found that juvenile *H. scabra* fed when moving, while burrowing and while emerging, but that they stopped ingesting sand when completely burrowed. These results are similar to those of Yamanouchi (1939, 1956) who observed that food transit in adults occurred mainly when they were on the surface. Similarly, Anonymous (1978b) noted that *H. scabra* generally came out of the sand for feeding in the Andaman Islands. Yamanouchi (1939, 1956) also observed that the intestinal content decreased when individuals were in the burrowing stage. More precisely 52% of the intestine was full of sediment in recently burrowed individuals, the value dropped to 32% when animals were burrowed, and increased to 87% when individuals were on the surface.

Mercier *et al.* (1999a) found that the marked daily cycle and the intermittent digestive transit render intestinal indices unsuitable markers for in situ investigations of feeding status. This inadequacy may explain why Wiedemeyer (1992) obtained variable intestinal indices in individuals of different drained body weight collected from the field in Japan, leading him to conclude that *H. scabra* fed during the night when burrowed and spent 8 h during the day without ingesting any sediment. He specified that small individuals had two short feeding periods, corresponding to the highest intestinal index values found after sunset and before sunrise (Wiedemeyer, 1992). The earlier studies of Yamanouchi (1939, 1956), which were mostly based on field observations of adults, more closely resemble the behaviour observed by Mercier *et al.* (1999a, b): *H. scabra* began to burrow between 0200 h and 0400 h, at which time they stopped eating and were not very active, emerging again between 1200 h and 1800 h. Yamanouchi (1939, 1956) also pointed out that the cycle was not perfectly correlated with light and that the factors regulating it would be complex. In fact, the cycle of adults is likely to be similar to that observed in larger juveniles and thus dependent on temperature variations (Mercier *et al.*, 1999a).

The highest feeding rate, 19.8–79.1 mg dw (dry weight) min^{-1}, was measured during the emerging behaviour of juveniles, and the maximum intestinal transit of 2.0–4.5 mm min^{-1} occurred just a few moments later. Complete passage of ingested material through the gut took between 30 and 60 min (Mercier *et al.*, 1999a). Yamanouchi (1939) estimated the time needed for the gut to fill was around 2 h for an adult. However, Wiedemeyer (1992) estimated that the transit was considerably lower at 2.6 cm h^{-1}. Following digestive transit, faeces are ejected from the anus in characteristic balloon-shaped strings. The egestion rate of material is irregular (Yamanouchi, 1939).

It is generally believed that feeding *H. scabra* play an important role in bioturbation (Massin, 1982, Wiedemeyer, 1992). Maximum feeding rates in juvenile *H. scabra* between 40 and 140 mm length were around 60–80 mg dw min^{-1} (Mercier *et al.*, 1999a). This would yield a daily ingestion of approximately 115 g dw sediment, or an annual intake of over 41 kg dw for a single individual. However, if the average feeding rate recorded during the active hours is used, we obtain a value of 22.1 ± 3.3 mg dw min^{-1}, a daily ingestion of about 32 g dw sediment or <12 kg dw processed annually. Other studies on adult *H. scabra* reported *in situ* values of 196 g dw ingested by an individual every day (Yamanouchi, 1939).

Battaglene *et al.* (1999) indicated that ca. 10% of the total weight of juveniles could be attributed to sand content in the intestine. The total amount of material found in the digestive tract could reach 5 g in adults and the pH level in a stomach full of material was around 6 (Yamanouchi, 1939). Wiedemeyer (1992) stipulated that *H. scabra* ingested between 23 and 31% of their drained body weight every day. The assimilation of organic matter varied between 0.13 and 0.18%. Considering that the material ingested by different sizes of individuals varied and that feeding behaviour was not a constant process, Mercier *et al.* (1999a) recommended that calculation of the assimilation of organic material should be performed using the period when the sea cucumber is on the surface. The quantity of organic matter was estimated by Baskar (1994) to be 2.1% in the oesophagus, 2.3% in the stomach and 1.7% in the intestine, suggesting that most absorption occurred in the stomach. Wiedemeyer (1992) and Mercier *et al.* (1999a), both indicated that *H. scabra* ingested their own faecal pellets. Wiedemeyer (1992) noted that there was 21% more organic matter in the pellets than in the surrounding sediment, whereas Mercier *et al.* (1999a) found that the faecal material of *H. scabra* was alternatively enriched and depleted compared with surrounding sediment over their daily cycle, depending on whether or not they were actively feeding. In fact, all components of feeding – including foraging activities, locomotive speed, feeding rate, intestinal transit, intestinal index and organic matter absorption efficiency – were discovered to be dependent upon the time of the day (Mercier *et al.*, 1999a). Organic content was usually highest in the last third of the intestinal tract toward the end of the burrowing cycle, e.g. after 6 h spent burrowed or while emerging and settling on the surface. Organic content was typically higher in the first third of the intestine than in the last third during the feeding hours (Mercier *et al.*, 1999a). Wiedemeyer (1992) found that the organic matter content of the posterior intestine in adult *H. scabra* was enriched by 7% compared with surrounding sediment. However, his data were obtained from the combined results of 476 individuals of various sizes collected on different

dates at different times during the day, without consideration for the marked daily cycle.

As discussed earlier in the substratum selection section, Mercier *et al.* (1999a, b) demonstrated that *H. scabra* were able to choose between different types of substrata: when given a choice, *H. scabra* preferred sand and muddy sand. Moreover, *H. scabra* was able to identify sediments containing higher organic content. Some believe that *H. scabra* are able to discriminate between sediments on the basis of grain size (Wiedemeyer, 1992). For instance, Baskar (1994) found that gut contents of adult *H. scabra* collected from the wild were dominated by muddy grains between 125 and 250 μm in diameter. His conclusions that *H. scabra* feeds selectively can be debated, as he did not compare gut contents with surrounding sediment. His findings reflect a substratum preference but not necessarily selectivity within a given substratum. Wiedemeyer (1992) indicated that the feeding mode of the different size classes of *H. scabra* varied mainly with the time of digestive transit, feeding cycles, particle size and organic matter assimilation.

14. PHYSIOLOGY, TISSUE BIOCHEMISTRY AND BIOTOXICITY

14.1. Physiology

Very few data exist on the physiology of *Holothuria scabra*. Using a differential gas-volumeter method Mukai *et al.* (1990) established that *H. scabra* had oxygen/nitrogen ratios ranging from 19.3 to 44.2, and that the total energy demand for respiration was 6.56 to 12.29 Kcal d^{-1}.

14.2. Biochemistry

As described in Table 10, the gross chemical composition of *H. scabra* is fairly well known (Springhall and Dingle, 1967; Sachitananthan, 1972). Krishnan and Krishnaswamy (1970) and Krishnan (1971) established the sugar pathway transport in different tissue compartments showing the key role of the haemal system, amoebocytes and coelomocytes. Anjaneyulu *et al.* (1995) described a new steroid glycoside from *H. scabra*. *H. scabra* possess a variety of carbohydrates, 1-2-glycol groups, acid mucopolysaccharides and muco-glycoprotein in their tissues (Krishnan, 1968). Sarma *et al.* (1987) isolated and described two sapogenin compounds and two glycosides from Indian specimens. Elanganayagam *et al.* (1982, 1983) and Mahendran *et al.* (1983) discussed the saponin and holothurin content,

Table 10 Composition (%) of *Holothuria scabra* according to six different studies conducted in different countries.

Composition	Australia[a]	Egypt[b]	India[c]	India[d]	India[e]	India[f]
Moisture	1–4.9	27	27.1	15	27	22
Crude protein	31.5–58	43	43.1	65	43	35–82
Fat	1.2–2	2	2.2	1	2	Traces
Fibre	–	–	–	–	–	–
Ash	43.7–79.9	–	27.6	11	21	15–30
Calcium $100\,g^{-1}$ tissue	6–12	–	1.2	–	–	–
Phosphorus $100\,g^{-1}$ tissue	0.1	–	0.7	–	–	–
Sodium $100\,g^{-1}$ tissue	5.2	–	2.5	–	–	–
Potassium $100\,g^{-1}$ tissue	0.6	–	–	–	–	–
Iron $100\,g^{-1}$ tissue	–	–	0.16	–	–	–
Insoluble ash	–	7	7	<0.5	7	–
Energy Cal kg^{-1}	2050	–	–	–	–	–
Minerals	–	21	–	–	–	–

[a]Springhall and Dingle (1967), [b]Din (1986), [c]Sachithananthan (1972), [d]Durairaj (1982), [e]Shenoy (1977), [f]Jacob (1973).

while Stonik *et al.* (1998) identified the free sterol composition of *H. scabra*. Ridzwan *et al.* (1995) screened for antibacterial agents in specimens from Sabah and Kobayashi *et al.* (1991) reported several antifungal oligoglycosides. Springhall and Dingle (1967) experimented with a diet based on boiled *H. scabra* and studied its pathology on the growth of chickens. Elanganayagam *et al.* (1980) studied the nutritional composition of *H. scabra* tissue.

14.3. Biotoxicity

Although very few studies have examined tissue toxicity, Halstead (1965) established that *H. scabra* was a toxic sea cucumber. Rao *et al.* (1985a, b) noted that extracts from the body wall and viscera of *H. scabra* inflicted distress, loss of equilibrium and death in fish. In addition, tests on mice induced immediate reversible paralysis.

15. PREDATORS

D. B. James *et al.* (1994a) and MPEDA (1989) indicated that the main predators of the larval forms of *Holothuria scabra* were copepods and ciliates that attacked the larvae, causing injury and death. These organisms also harmed juveniles, especially the newly settled ones, by indirectly competing for food (Battaglene, 1999a, 2000, Battaglene *et al.*, in press). To control copepods and ciliates, D. B. James *et al.* (1994a) added chemicals containing organo-phosphorus to rearing tanks. Copepods were killed with 2 ppm dipteryx in 2 h with no harmful effect on sea cucumber larvae. Mercier *et al.* (2000a) observed that tectibranchs and some species of amphipods were feeding on pentactula larvae and newly settled juveniles in the laboratory. Recent experiments in the Solomon Islands showed that 30–100% of juvenile *H. scabra* of 20–70 mm released on sand near coral reefs were eaten within 24 h by a variety of fish (including Lethrinidae, Balistidae and Nymipteridae) whereas there was no predation of juveniles of the same size released on subtidal sandy substrata near mangrove forests and seagrass beds well away from coral reefs (Dance *et al.*, in press).

Although *H. scabra* is known to be toxic (Bakus, 1968; Stott *et al.*, 1974; Rao *et al.*, 1985a, b), the fact that small juveniles forage only at night seems to suggest a possible predator avoidance measure. Adults were never observed to be preyed upon in the field (Mercier *et al.*, 2000b).

16. DISEASES

Morgan (2000c) observed that *Holothuria scabra* brood stock collected in October 1997 became diseased after a few weeks in captivity. The first visual signs, pigmentation loss and presence of copious amounts of mucous, coincided with a period of weight loss. Although the most common bacterium present on diseased individuals was *Vibrio harveyi*, there were also low numbers of a motile gram-negative rod bacteria, and the exact nature of the infection is still unknown. Surface damage to the epidermis was not a prerequisite for infection, but infection progressed more rapidly from an existing wound. Most infected animals died within 3–7 days (Morgan, 2000c). A similar condition was noted in juveniles grown in captivity in the Solomon Islands (S. C. Battaglene, personal communication).

17. ASSOCIATION WITH OTHER SPECIES

Holothuria scabra adults provide shelter to numerous species of crustaceans, gastropods, worms and fish, which live on their external surface or inside their coelom or respiratory trees (Table 11). Most of the animals associated with *H. scabra* were not found to affect adversely their hosts, although minor injuries to the respiratory trees have been recorded (Jones and Mahadevan, 1965; Van den Spiegel *et al.*, 1992). Hamel *et al.* (1999) observed respiratory tree atrophy and gonadal reduction in *H. scabra* parasitized by the pea crab *Pinnotheres halingi* in the Solomon Islands.

In New Caledonia, from a sample of over 300 *H. scabra versicolor*, only six hosted the following carapid fish: one individual with one *Carapus mourlani*, three individuals with one *Encheliophis vermicularis* each and two individuals with two *E. vermicularis* (Conand and Olney, unpublished data).

18. FISHERIES

Holothuria scabra is usually categorized as a first grade product on the international beche-de-mer market (Arakawa, 1991; Conand and Byrne, 1993; South Pacific Commission, 1994, 1995; Baine and Sze, 1997; Conand, 1999b), where it fetches some of the highest prices (Conand and Sloan, 1989; Adams, 1992). Consequently, *H. scabra* is among the most

Table 11 Description of the commensal, parasitic and symbiotic associates of *Holothuria scabra*.

Species	Geographic location	Observations	References
Vibrio sp. (Bacteria)	Indonesia	On the body wall	Zafran, 1992
Hapalonotus reticulatus (Crustacea)	Papua New Guinea and the Solomon Islands	In respiratory tree	Van den Spiegel and Ovaer, 1991; Van den Spiegel *et al.*, 1992; Hamel *et al.*, 1999
Pinnotheres halingi (Crustacea)	Solomon Islands	In respiratory tree	Hamel *et al.*, 1999
Pinnotheres deccanensis (Crustacea)	India	In respiratory tree, cloaca, rarely in coelomic cavity	Chopra, 1931; Jones and Mahadevan, 1965; Adithiya, 1969; Jangoux, 1987; D. B. James, 1987b, 1989b, 1995c
Pinnotheres semperi (Crustacea)	Singapore	–	Lanchester, 1900
Periclimenes imperator (Crustacea)	Papua New Guinea	–	Van den Spiegel *et al.*, 1992
Lissocarcinus orbicularis (Crustacea)	Papua New Guinea, Madagascar and the Solomon Islands	On the body wall and in the cloaca	Crosnier, 1962; Van den Spiegel *et al.*, 1992; Hamel *et al.*, 1999
Copepods (Crustacea)	–	–	Humes, 1980
Lichothuria mandibularis (Crustacea)	Madagascar	–	Jangoux, 1987
Mucronalia sp. (Gastropoda)	Mozambique	Gall around the anus	Macnae and Kalk, 1962; Bakus, 1973
Prostilifer sp. (Gastropoda)	India	–	D. B. James, 1998c
Odostomia sp. (Gastropoda)	Mozambique	Attached to the buccal membrane or to the body wall	Macnae and Kalk, 1962; Bakus, 1973
Encheliophis (*Jornanicus*) *gracilis* (Osteichthyes)	Solomon Islands and elsewhere in West Pacific and Indian Oceans	Coelomic cavity	Hardy and Cowan, 1967; Jangoux, 1987; Hamel *et al.*, 1999
Encheliophis vermicularis (Piscea)	–	–	Murdy and Cowan, 1980
Carapus sp. (Osteichthyes)	Papua New Guinea	In respiratory tree	Van den Spiegel *et al.*, 1992

intensively exploited holothurians in the Indo-Pacific (Gonzales, 1975; Bruce, 1983; Sloan, 1984; Van Eys, 1986, 1987; McElroy, 1990; Preston, 1990a; Valayudhan and Santhanam, 1990; D. B. James and Ali Manikfan, 1994; Sachithananthan, 1994a; Taylor-Moore, 1994; Conand, 1997b, 1999a, b), although Van Eys and Philipson (1991) state that import statistics are unreliable. The most recent data on the world trends are presented in Conand (1998a, 1999a, in press) and Conand and Jaquemet (in press). In 1996, approximately 23 000 mt of processed product were derived from the family Holothuriidae. While it is generally possible to extrapolate these data to species where fisheries are more or less monospecific, as is the case in temperate areas, it is not possible in multiple species Indo-Pacific fisheries. Another difficulty is the change of target species, which occurred during the last decade. It is probable that the first grade *Holothuria nobilis, H. fuscogilva, Thelenota ananas* are overexploited everywhere and that the capture data are mostly referable nowadays to *H. scabra, H. scabra versicolor* and *Actinopyga* spp. Aside from being exported, *H. scabra* is also consumed locally in Fiji and Papua New Guinea (Adams, 1992; Conand and Byrne, 1993). From 1989, coinciding with the declining copra prices, *H. scabra* has become the target species in numerous Indo-Pacific countries (Conand and Byrne, 1993).

18.1. History and price fluctuations

According to Hornell (1917) and D. B. James and Baskar (1994) the Chinese have been trading beche-de-mer with Southern India and Sri Lanka for about one thousand years, anciently in exchange for porcelain, silk and sweetmeats. As *H. scabra* is one of the most common species along these coasts, there is a good chance that their harvesting originated from that period. Carter *et al.* (1997) noted that Macassan sailors, aboriginal people from Indonesia, were fishing *H. scabra* in the 1600s. Today, *H. scabra* is exported to the Asian market via Hong Kong and Singapore (Conand 1989; Conand and Byrne, 1993; Sachithananthan, 1994a) and dominates the market (Sachithananthan, 1994b).

Prices of dried *H. scabra* have fluctuated over the past decades. While *H. scabra* was considered a low value species 15 years ago, its current trade value is among the highest on the market (Holland, 1994b). The selling price of *H. scabra* was US\$0.32 kg^{-1} in 1972 (Sachithananthan, 1972). D. B. James (1986c, 1989b) noted that *H. scabra* was no more than fourth on the market in 1986 when it was sold for US\$0.38–0.70 kg^{-1}. In the early 1990s *H. scabra* was the most common medium value beche-de-mer, and later the price for *H. scabra* beche-de-mer was US\$14–20 kg^{-1} (Sommerville,

1993). Prices peaked at US$50–100 kg^{-1} in recent years (Mercier and Hamel, 1997; Conand, 2001). In some areas, such as the Andaman and Nicobar Islands and elsewhere in India, fishermen devoted 90% of their effort to *H. scabra*, leaving behind other valuable species (D. B. James, 1994c, h; D. B. James and P. S. B. R. James, 1994). Owing to this monospecific fishery, stocks of *H. scabra* have declined rapidly (P. S. B. R. James and D. B. James, 1994a). For instance, in Fiji, the catch of *H. scabra* decreased by 80% between 1979 and 1993 (Stewart, 1993).

18.2. Harvesting techniques

Face masks or goggles are universally worn for collection of *H. scabra*. However, when large numbers of sea cucumbers can be found in shallow water on the sand or muddy sand flats, they are collected by hand during low tide (MPEDA, 1989). In some places in India, women and children collect *H. scabra* during low tide, while men focus their efforts in deeper water (Radhakrishnan, 1994). In Sri Lanka and Tanzania, fishermen use steel-pronged forks mounted on long handles (D. B. James, 1989b). Scuba diving equipment is used when the only individuals available are in deep water, such as in Sri Lanka, where fishermen dive to collect *H. scabra* at depths between 6 and 20 m (Anonymous, 1978a). Fishermen in the Solomon Islands harvest deep specimens with a straightened fish hook inserted in a piece of lead of about 3 kg, attached to a fishing line (Kanapathipillai and Sachithananthan, 1974), but these practices are not recommended as they damage the skin and give lower grade products (Conand, 1999b). In India, *H. scabra* are sometimes dredged by sail boats concurrently with fish and prawns (MPEDA, 1989).

Sachithananthan (1986) indicated that *H. scabra* kept out of water for long periods will suffer skin ruptures, and, if packed in irregular containers, their body wall will take the form of the container. They thus suggest that plastic boxes with a smooth interior should be chosen for stocking and transport. Sachithananthan (1986) also reported that freshly collected sea cucumbers will eject 2–3 cm of silt from the anus, and this is a proper time to apply a pressure on the anal region to stimulate evisceration.

18.3. Processing into beche-de-mer

Specific methods have been developed to process *H. scabra* and *H. scabra versicolor* in order to remove the chalky spicules from their body wall. The

main steps involve making a small incision in the posterior area across the anus (20 mm long); evisceration of the internal organs; a first boiling for a few minutes; burying in sand for about 18 h (to remove the chalky epidermis); cleaning; a second boiling for 45 min; and finally drying in the sun and packing (Hornell, 1917; Adithiya, 1969; Baird, 1974; Anonymous, 1978a; Durairaj, 1982; Tebchalerm, 1984; D. B. James, 1986b, 1989b; Sachithananthan, 1986, 1994a; Sachithananthan *et al.*, 1975a; Conand, 1989, 1990, 1994, 1999b; Preston, 1993; Gurumani and Krishnamurthy, 1994; D. B James and P. S. B. R. James, 1994; South Pacific Commission 1994, 1995; Nair *et al.*, 1994). Sometimes, part of the drying can be done by smoking to suit the needs of buyers (South Pacific Commission, 1994, 1995).

During the processing, specimens decrease in size and weight considerably (Conand, 1979, 1990; Preston, 1990b; Vuki, 1991). Baskar and James (1989) measured a loss of ca. 42% in size and ca. 91% in weight. Durairaj *et al.* (1984) stated that the shrinkage percentage was between 50 and 60%. Shelley (1985b) found that final dry weight corresponded to 5% of original wet weight and Conand (1990) noted that dried *H. scabra versicolor* only represents 6% of the fished individual in weight and 38% in length. The South Pacific Commission (1994, 1995) also noted that beche-de-mer from *H. scabra* corresponded to ca. 5% of initial weight, e.g. from an initial weight of 370 g, the dry product decreased to about 20 g. About 8–12 large individuals must be processed to produce 1 kg of beche-de-mer measuring 10–15 cm (South Pacific Commission, 1994, 1995). Holland (1994a, b) also noted that up to 20 beche-de-mer produced with *H. scabra* were needed to obtain 1 kg of product in the Solomon Islands. Conand and Tuwo (1996) indicated that *H. scabra versicolor* with a fresh length of 34 cm will give 13 cm beche-de-mer.

The processing of beche-de-mer has a major influence on the price. The main defects or faults that can influence the quality of the final product are: insufficient drying, scratches on the body wall and unattractive incisions. Also, very small beche-de-mer or non-uniform products are less appreciated and are associated with an inconsistent processing method (South Pacific Commission, 1994, 1995; Conand, 1999b). Conversely, a sizable product with a proper appearance, no bad odour, an appropriate colour and under 20% moisture will have a good value (Kanapathipillai and Sachithananthan, 1974). Sachithananthan (1994a) noted that the muscular body bands should be preserved during processing. The product is graded according to size, with larger animals fetching a higher price.

Nair *et al.* (1994) indicated that a good beche-de-mer should be properly dried, free from fungal, insect and mite infestation, and suggested improvements in processing techniques to avoid these problems. In

order to remove the chalky deposits resulting from imperfect boiling, Sachithananthan *et al.* (1975b, 1979) developed a de-scummer in Sri Lanka. Peranginangin *et al.* (1994) described the use of enzymatic treatment with papaya leaves that gave the best results to remove lime deposits from smoke-dried sea cucumbers. Individuals were sometimes frozen before processing and Yunizal *et al.* (1997) studied the impact of this step on the final quality of beche-de-mer.

18.4. Catches

Data on holothurian fisheries are abundant, although they often refer to all commercial species, and very few specific data on *H. scabra* are available. Conand (1989) indicated that a single diver could collect between 83 and 230 kg h^{-1} of *H. scabra*, and between 47 and 77 kg h^{-1} of *H. scabra versicolor*. Anonymous (1993) noted that *H. scabra* represented ca. 28% of the New Caledonia catch of sea cucumbers with ca. 393 000 kg yr^{-1}. An estimated 100–150 tons yr^{-1} are collected in north-west Sri Lanka (Anonymous, 1994; D. B. James and Baskar, 1994). D. B. James (1973) mentioned that 2.4–3.5 kg per boat per day could be caught at times in India. Anonymous (1996a) indicated that the annual catch of *H. scabra* was 2089 kg in Tonga. The total catch of *H. scabra* on the east coast of Queensland was ca. 48 000 kg between July 1995 and June 1996 (Anonymous, 1997a). In New Caledonia, species categories were introduced into fisheries statistics in 1985 to obtain a better evaluation of the fishery. Unfortunately *H. scabra* and *H. scabra versicolor*, which were not of high value at that date, were classified as "others". Nevertheless, given the demand for sandfish in the recent years, it is reasonable to assume that this category is dominated by *H. scabra*. In recent years this proportion of the catch, in metric tons, has been: 13.7 (in a total of 24) in 1998, 32.7 (in a total of 46.7) in 1997, 13.3 (in a total of 33.6) in 1996, 34.9 (in a total of 47.9) in 1995 (Conand, unpublished data).

Depletion of *H. scabra* stocks by overfishing has been demonstrated in New Caledonia (Conand, 1989) and in Malaysia (Forbes and Ilias, 1999). In India, catches reached 91 tons in 1975 and dropped to 11 tons in 1985 (D. B. James, 1989b). Similarly, Lokani *et al.* (1995b) and Lokani (1996) indicated that harvests reached 192 000 individuals in 1991 and dropped to 39 000 in 1993 in Papua New Guinea. The yield estimate per hectare also decreased sharply between 1991 and 1993. Stocks of *H. scabra* also declined drastically in Mozambique, as a result of intense exploitation (Abdula, 1998). Today, fishing is prohibited in many areas until the sea cucumber population is replenished.

18.5. Management

Battaglene (1999a, b, 2000), Battaglene *et al.* (in press) and Ramofafia *et al.* (1999, in press) indicated that overfished populations of *H. scabra* could take decades to recover if harvesting continues, unless new ways to protect or manage the stocks are implemented. Developing countries often do not have the resources to enforce restrictions on catches, and social and coastal tenure systems often complicate the application of regulations (Conand, 1989, 1990, 1994; Conand and Sloan, 1989; Ambrose Fernando, 1994; Lokani, 1995a; Battaglene and Bell, 1999). P. S. B. R. James and D. B. James (1994b) also suggested that *H. scabra* are likely to be depleted unless conservation measures are adopted. The general lack of fisheries management is reflected by the paucity of documented regulations (Conand and Sloan, 1989). Overall, because of the unreliable fisheries data and the poor control over the actual catches, it is difficult to construct analytical models to manage the resource (Conand, 1989, 1990, 1994; Conand and Sloan, 1989).

Considering the drastic decline of most sea cucumber populations, including *H. scabra*, Conand (1989, 1990, 1994), Adams (1993), Holland (1994b), Lokani *et al.* (1995b) and Lokani (1996) proposed several measures to protect the resource: (1) establishing a minimum size limit for capture; (2) introducing strict quotas; (3) limiting the number of export businesses with strict quotas; (4) alternating closed seasons; (5) banning scuba diving or specialized apparatus for harvesting; (6) establishing reserves and (7) promoting stock enhancement.

The size at collection is an important issue in many regions. Conand and Tuwo (1996) indicated that juvenile *H. scabra* ca. 10 cm long were collected on the shore at low tide in Indonesia. Lokani *et al.* (1995a) and Lokani (1996) proposed that the management regime in Papua New Guinea should include a closed season during the reproductive period and a catch limited to ca. 49 tons composed of individuals not smaller than 21 cm. Baskar and James (1989) and P. S. B. R. James and D. B. James (1994a, b) also suggested that banning the export of beche-de-mer under 75 mm and introducing a closed season each year could help preservation of natural stocks in India. They also indicated that the harvestable size of *H. scabra* should be between 130 and 340 mm in length in India. The minimum size should be based on the mean size at first maturity of the species. It has been increased in New Caledonia, at 185 g (live weight) or 16 cm (total length) for *H. scabra* and 490 g or 22 cm for *H. scabra versicolor* (Conand, 1989, 1994). Without a moratorium or ban on the fisheries of *H. scabra* in Fiji, the global exports were expected to drop by 80%, but a 5 yr ban followed by a 5 yr fishing period could maintain a sustainable fishery (Adams, 1992). However, this information was

published in the early 1990s and there is now a total ban in place in many Pacific island nations including Samoa and the Solomon Islands (S. C. Battaglene, personal communication).

Catch quotas should be based on evaluations of the maximum sustainable yields (MSY). The values estimated for New Caledonia are 31 kg ha^{-1} yr^{-1} for *H. scabra* and only 11 kg ha^{-1} yr^{-1} for *H. scabra versicolor* (Conand, 1989, 1994).

D. B. James (1985) noted that the creation of a national marine park was proposed in the Gulf of Mannar, southern India, which will protect *H. scabra* among other species. A similar project has been developed in the Solomon Islands with the Arnavon Marine Reserve that protects several species, including *H. scabra* (Lincoln Smith *et al.*, 1997). *H. scabra* is also protected in the Marine National Park of the South Andaman, India (D. B. James, 1991). However, close monitoring is necessary, as many cases of illegal fishing are reported worldwide in protected zones (Conand, 1997a, 1998b).

Even though many good ideas have been promulgated, the tropical fishery for beche-de-mer remains a generally unregulated enterprise, under poor control and surveillance. The Government of Papua New Guinea imposed a partial moratorium to protect the endangered resource, but Lokani *et al.* (1995b, c) indicated that the size limit of 15 cm and the gear restrictions did not prevent overfishing. Illegal fishing was observed in the protected waters of the Torres Strait, Australia (Lokani *et al.*, 1995b). Similarly, although the government of the Solomon Islands banned the collection and sale of *H. scabra* in 1997 (Battaglene, 1998a), illegal harvesting and commerce continues. The government of India imposed a ban on the export of the sea cucumbers under 75 mm in 1982 (Silas *et al.*, 1985; P. S. B. R. James and D. B. James, 1994b), but created a crisis in the industry, pushing forward the idea of hatchery and stock enhancement (D. B. James, 1994h). Gurumani and Krishnamurthy (1994) indicated that in some areas of India fisheries are restricted to waters between 2 and 5 m depth, as the juveniles are located in shallow coastal water. Current protective measures in Yemen include the minimal legal fresh size limit of 10 cm and restriction of the harvesting season to a few months during summer (Gentle, 1985). Uthicke and Benzie (1998) indicated that *H. scabra* is subject to export control under the Wildlife Protection Act 1982 in Australia. The 10 yr moratorium on the export of sea cucumbers in Tonga is effective and there are reports of substantial recovery by several species (I. Lane, personal communication).

During a recent meeting on conservation of sea cucumbers in Malaysia, Hashim *et al.* (1999), "the Malaysian Network for Holothurian Conservation", added several important points for management of

holothurians, such as establishing conservative management guidelines and baseline surveys prior to the start of a fishery. The management plans also proposed a ban on harvest during the breeding season, the introduction of quotas and minimum landing sizes, the establishment of permanent survey sites and preserved areas, the maintenance of records on harvesting data and a ban on scuba equipment. Over recent years, numerous countries have increased interventions to protect the resource by means of different approaches (Hashim *et al.*, 1999).

A regional concern was also raised in the western Indian Ocean during the course of the Environmental Programme of the Indian Ocean Commission (COI/EU) (Conand, 1999b). In Madagascar, several actions have taken place including the setting up of the National Trepang Traders Group (ONET), several national meetings to exchange information between the different participants in the "Beche-de-Mer Fishery System". This system includes fishermen, processors, traders, administrations and scientists, and involves various projects (Conand *et al.*, 1997, 1998).

The sustainable management of holothurian fisheries requires production models that combine data on fishery activity, on population dynamics and on socio-economic aspects that are particularly important for these small artisanal activities. The paucity of data on catches, as well as on biomass, is the main reason why management generally does not exist on a sustainable basis. A plan of action for the management of holothurian fisheries has been proposed by Conand (in press). It includes four components: assessment of stocks, improvement of statistics, improvement of collecting and processing procedures, and farming experiments (Table 12).

19. AQUACULTURE

The increasing demand for beche-de-mer, the drastic decline of natural populations due to overfishing, the corresponding decline of harvests and the high value of *Holothuria scabra* on the beche-de-mer market have promoted interest in aquaculture programmes in numerous countries. In fact, *H. scabra* is perceived as one of the best sea cucumber candidates for aquaculture (Battaglene and Bell, 1999; Conand, 1999b; Jangoux *et al.*, 2001). A summary by Pitt (2001) outlines most of the methods used for breeding and rearing *H. scabra*. To date, the research has focused on the development of methods for producing juveniles in hatcheries so that the potential for farming, restocking and stock enhancement can be assessed.

Table 12 Plan of action for the management of *Holothuria scabra*.

Fishery system	Management actions
Natural resources in commercial holothurian species	• Research on biology and stock assessment • Hatcheries – production of juveniles • Sea ranching – mariculture
Fishermen catches collected by wading, snorkling, scuba diving	• Respect of fishery legislation: size (ban on juveniles), periods, zones, national legislation • Collection of standardized statistics • Education
Processing by fishermen or processors	• Improving the quality during all phases of processing • Storage, grading • Education
Fishery services and customs, national, and international trade	• Communication between the agencies • Storage, grading • Standardized statistics • Access to information
Import and consumption	• Information on market regulations and preferences

From Conand (in press).

However, there have also been some grow-out trials of wild caught specimens in India and Indonesia.

19.1. Collection and maintenance of brood stock

The success of hatchery production of any species depends on the health of the brood stock (D. B. James *et al.*, 1994a) and the timing of collection with respect to the natural spawning periods (MPEDA, 1989; D. B. James, 1994h; D. B. James *et al.*, 1994a; P. S. B. R. James, 1996). Battaglene (1999a, 2000) and Battaglene *et al.* (in press) mentioned that collection of brood stock needed to be performed with minimal temperature and salinity variations to avoid evisceration during transport. A daily renewal of water or a running seawater system are the most suitable holding conditions. About 20–30 adults can be maintained in a 1 ton tank with 15 cm of sand and with fresh algae added once a week (Battaglene, 1999a, 2000; Battaglene *et al.*, in press). The sand in the tank is necessary to allow sea cucumbers to burrow (MPEDA, 1989). Hatchery sites should be located near the shore, with a source of unpolluted sea water, free of suspended particles, salinity should be between 30 and 40, and the area

should not be influenced by monsoon freshwater run-offs (D. B. James *et al.*, 1994a). Much care has to be taken to hold a good brood stock. For instance, Morgan (2000c) observed that some individuals maintained in holding tanks lost weight and presented a discoloration of the epidermis that could be a result of bacterial infection by *Vibrio harveyi*. Furthermore, the number of spawned oocytes and the hatch rate of eggs were found to decrease in brood stock maintained in captivity for more than a month (Morgan, 2000b, in press a).

In trials in India, spontaneous spawning occurred in >60% of *H. scabra* brood stock when shrimp pellets were added to promote bacterial production on the sediment (P. S. B. R. James, 1996). Spawning peaked in October but continued until January. Brood stock were also conditioned to gonadal maturity in captivity using a mix of soybean powder, rice bran, chicken manure, ground algae and prawn head waste (P. S. B. R. James, 1996). More details on spawning induction are provided in earlier sections.

19.2. Larvae and juvenile rearing

H. scabra were first produced in the laboratory in India. Rearing trials of *H. scabra* began in 1983 (D. B. James, 1983), but concrete results were achieved only in 1987 with successful induction of spawning and development of juveniles (D. B. James, 1994h). The techniques have been used or experimentally tested (with minor to substantial modifications), in Australia, Indonesia, the Maldives and the Solomon Islands, as well in other sites in India (Martoyo *et al.*, 1994; D. B. James, 1995b, 1998a, b; D. B James *et al.*, 1995; Morgan, 1996; Anonymous, 1997b; Battaglene, 1997, 1999a, c, d, 2000; Battaglene and Seymour, 1998; Battaglene *et al.*, in press). Generally, oocytes are rapidly removed from the spawning tanks, and washed several times to remove the excess sperm that might pollute the water and induce development of deformed embryos (MPEDA, 1989; Battaglene, 1999a, 2000; Battaglene *et al.*, in press). Optimal oocyte density has been determined to be 0.1 oocyte ml^{-1} under static conditions, whereas acceptable larval density was set at 0.1–0.4 larva ml^{-1} (Battaglene *et al.*, 1998, in press; Battaglene, 1999a, 2000). Alternatively, an optimal stocking density of larvae was estimated to be between 300 and $700 l^{-1}$, or 3 750 000 larvae in a 750 l tank (D. B. James *et al.*, 1994a). Battaglene (1999a, 2000) and Battaglene *et al.* (in press) kept the oocytes and larvae in suspension by slight aeration. D. B. James (1994h) and D. B. James *et al.* (1994a) stated that the water of the rearing tank was cleaned every 3 or 4 d by sieving the larvae on

80 μm mesh. The same authors mentioned that the larvae needed to be fed by adding 20 000–30 000 algal cells ml^{-1} (*Isochrysis galbana* or *Chaetoceros* sp.). Non-chained algae were apparently preferable (D. B. James, 1994h; D. B. James *et al.*, 1994a). Manikandan (2000) used a combination of *I. galbana*, *Dunaliella salina*, *Pavlova lutherii* and *Tetraselmis chuii*. Other larval diets have been discussed earlier in the feeding section. Sipahutar and Soeharmoko (1989) stated that *H. scabra* juveniles develop well in water filled with sandy clay soil in which the seagrass *Enhalus* grows.

Battaglene *et al.* (1999) demonstrated that mortality was high after settlement, with only ca. 34% survival in the laboratory. In Indonesia, similar survival rates under aquaculture conditions were also achieved by Rachmansyah *et al.* (1992) and Muliani (1993). D. B. James *et al.* (1994a) noted that survival was largely influenced by the quality of the water used and the density of competing organisms present. The same authors determined that the temperature should be maintained between 27 and 29°C, dissolved oxygen between 5 and 6 $ml\,l^{-1}$, and pH between 7.5 and 8.6. The lethal salinity was 12.9 p.s.u. and optimal salinity was between 26.2 and 32.7 p.s.u. (D. B. James *et al.*, 1994a). Suboptimal salinities negatively affected the normal development, resulting in high proportions of deformed larvae and high mortalities. Ammoniacal nitrogen should be <70 to 430 $mg\,m^{-3}$ (MPEDA, 1989; D. B. James, 1994h; D. B. James *et al.*, 1994a; P. S. B. R. James, 1996). Battaglene (1999a, 2000) and Battaglene *et al.* (in press) indicated that light intensity should be maintained around 400 lux during larval rearing.

Collection of newly settled juveniles for measurement can be done mechanically (P.S.B.R. James, 1996) or with the aid of 1% w/w potassium chloride (KCl) as a detachment agent, a technique determined to be less damaging and more efficient than mechanical removal with water (Battaglene and Seymour, 1998).

Following settlement, juveniles were fed with algal extracts, first filtered through 40 μm mesh, and, after 1 mo, filtered on 80 μm mesh (D. B. James *et al.*, 1994a). After 2 mo, fine algal powder was added to the food. Juvenile *H. scabra* can be reared in concrete tanks at densities of around 10 000 individuals per m^2 until they reach 10 mm in length (P. S. B. R. James, 1996). To optimize survival, between 200 and 500 individuals may be maintained on each m^2 of surface (D. B. James *et al.*, 1994a). Battaglene (1999b) found that juveniles survived better on hard substrata until they reach 20 mm in length and should be transferred to sand afterwards to optimize their growth. D. B. James *et al.* (1994a) and P. S. B. R. James (1996) successfully transferred laboratory-reared *H. scabra* to sandy substrata in the field when they reached between 10 and 25 mm in length.

19.3. Polycultures

A variety of experiments have examined the feasibility of rearing *H. scabra* within polycultures, with other animals or plants. Most projects were performed in Indonesia, where wild caught and transplanted juvenile *H. scabra* positively grew with the seaweed *Eucheuma cottonii* (Madeali *et al.*, 1993a, b), *Eucheuma* sp. (Rachmansyah *et al.*, 1992; Daud *et al.*, 1993; Tangko *et al.*, 1993b) or *Gracilaria* sp. (Daud *et al.*, 1991; Tangko *et al.*, 1993c). Battaglene (1999a, 2000), Battaglene and Bell (1999) and Battaglene *et al.* (in press), have suggested that there may be scope for rearing juvenile *H. scabra* in penaeid shrimp ponds as a way of mass producing the animals for restocking and stock enhancement. However, they stress that predation by the shrimp, and toxicity of *H. scabra* to shrimps, need to be evaluated first.

19.4. Stock enhancement

Battaglene (1998b, 1999a, b, 2000) and Battaglene *et al.* (in press) indicated that the release of juveniles produced in hatcheries is one way of rebuilding wild stocks, a process referred to in the literature as restocking or reseeding. Moreover, it is also possible to increase harvest beyond historical levels by releasing large quantities of cultured juveniles in the wild to reach the carrying capacity of the habitat, a process called stock enhancement (Battaglene and Bell, 1999). Battaglene and Bell (1999) determined that *H. scabra* has attributes that make it suitable for stock enhancement: its high value, its preference for circumscribed habitats, slow-moving habits, simple feeding regime, and its fairly well understood reproductive habits.

However, even if developing countries can implement sound management of sea cucumber stocks, it will take many years to restore depleted fisheries (Battaglene and Bell, 1999). Low cost hatchery techniques need to be developed to permit affordable rearing of larvae and juveniles, and researchers need to develop strategies to maximize the survival of individuals released in the wild (Battaglene and Bell, 1999).

D. B. James *et al.* (1994a) released 40 mm long *H. scabra* (about 2 mo old) in a 25 m^2 enclosed and controlled area in the field located in about 1.5 m of water. D. B. James (1994g) further indicated that ca. 500 juveniles between 65 and 160 mm in length could be stocked in enclosed areas of 1500 m^{-2}, where they were found to grow to 190–290 mm in length in 7 mo. Mercier *et al.* (1999c, 2000b, in press a) experimented with the release of laboratory-reared juveniles in different habitats with good survival rates and rapid growth. These data have been discussed in a previous section. More

recently, a Solomon Islands team performed the first large release of juvenile *H. scabra* in the field, thus taking a new step toward stock enhancement (S. C. Battaglene, personal communication; Dance *et al.*, in press). Moreover, projects on pond culture of *H. scabra* are planned for Vietnam, Madagascar and New Caledonia by ICLARM to assess the scope for polyculture and the mass rearing of juveniles for restocking and stock enhancement (J. Bell, personal communication).

20. CONCLUSIONS

In view of the present review and because the identification issue has posed a number of problems in the past, we encourage research teams working on *Holothuria scabra* to verify the identification of their specimens. Overall, we conclude that future research needs to focus on field monitoring, better understanding of natural predatory pressures, genetic variation and isolation of populations over the geographic ranges. Detailed study of the reproductive cycle and spawning cues will improve our understanding of the life history of *H. scabra*. Clarification of the status of *H. scabra versicolor* is also needed.

The accumulated knowledge about the biology of the species has paved the way for sustainable management of remaining populations through restocking and stock enhancement, and also offers the potential for increasing production through farming. Further investigations are welcomed given that *H. scabra* is one of the few tropical holothurians amenable to culture. However, fisheries statistics have to be improved to enable better management of remaining stocks and the use of marine protected areas needs to be examined. Finally, more active collaboration between and within countries of the Indo-Pacific should facilitate the gathering of knowledge on this valuable species.

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