Chapter I Integration of *Lates calcarifer* culture and *Cherax quadricarinatus* culture and constructed wetlands.

Abstract

A water use and re-use primary production system integrating *Lates calcarifer* (finfish) and *Cherax quadricarinatus* (crayfish) aquaculture species, and constructed wetlands planted with *Baumea articulata* or *Schoenoplectus validus* emergent plant species was constructed, trialed, and evaluated. The objectives of this experiment were (a) to integrate barramundi and red claw culture (in polyculture) and to produce and quantify successive animal crops of commercial relevance; (b) to integrate polyculture (see (a)) with constructed wetlands to test and quantify their application as in-line, discharge-water quality control systems, as well as secondary aquaculture medium; (c) to characterize the system as a whole in terms of a CNP mass balance, plant and animal growth efficiencies, aspects of biodiversity, and water quality and usage; and (d) to compare / contrast the performance of two emergent macrophyte species chosen for use in the polishing constructed wetland components of the system.

1.0 Introduction and Literature review

1.1 Aquaculture

The term 'aquaculture' describes the methods developed to farm aquatic organisms. Various forms of aquaculture have been practiced since its inception in China 3,000 to 4,000 years ago (Stickney, 1994, Black, 2001). A steadily increasing demand for fish and shellfish by the global population has developed, to meet a need for a rich protein source with a favorable taste, contributing to an over-exploitation of many of Earth's natural fishery ecosystems (Costa-Pierce, 1996). From 1986 to 1996, aquaculture has become one of the world's fastest growing agribusinesses (Costa-Pierce, 1996).

The percentage proportion of aquaculture revenue to Australia's total fisheries revenue increased from 18.9 to 29.2 % over the past seven years while in Queensland that increase was more dramatic (10.1 to 24.1 % over the same period) (Lobegeiger et al., 2001). Since 1988, salmon, pearl oyster, and edible oyster production have accounted for over 80% of the total revenue generated by Australian aquaculture operations (ANZECC, 2000). However, meat species such as barramundi and freshwater crayfish (animals cultured in this study) are becoming significant contributors to Australian agribusiness, and have produced over 8.8 and 5.7 million dollars value, respectively, in annual product in recent years. The value of Queensland's barramundi and red claw production was worth 4.9 and 0.8 million dollars in 1999-2000, respectively (Lobegeiger et al., 2001).

Of the 89 Queensland farms licensed to produce barramundi in 2000, 37

farms produced marketable fish that year (Lobegeiger et al., 2001). Cage culture in ponds or estuaries accounts for 75 % of grow-out operations, although there has been a large increase in the number of farms utilizing recirculating systems in Queensland; and in southern Queensland particularly there had been a large increase of smaller, heated, recirculating systems in 1999-2000 (Lobegeiger et al., 2001). From 1998 to 2000 barramundi fillets and whole fish product have increased in value from $ 13.57 to $ 17.43, and $ 8.49 to $ 9.13, respectively. In 2000 there were 12 Queensland red claw farms that produced ≥ 1 ton annual product: the 12 farms produced 95 % of Queensland's total production; 103 farms did not produce product that year (Lobegeiger et al., 2001). The value of red claw in Queensland has dropped steadily from $ 13.87 kg^{-1} (1993) to 11.79 kg^{-1} (2000) (Lobegeiger et al., 2001).

Through continual efforts to increase the amount of fish made available locally for consumption, aquaculture has become a diverse agribusiness practiced on commercial and subsistence levels in many global locations. The three main culture methods are employed by aquaculturists around the world include cage, tank, and pond production (both tank and cage production activities were part of this study). Cage production occurs in open water systems such as oceans, lakes, and rivers, and currently accounts for the majority of marine and freshwater finfish culture (Black, 2001). Wastes generated by cage culture are transferred directly into the host body of water where naturally occurring processes (e.g. dilution, sedimentation, and pelagic utilization) are the mechanisms of pollution control; and system design limits operator control over culture conditions and maximizes transfer of biochemical, pathogenic, and non-wild fish genetics (i.e. fish escape) into the environment. Capital and maintenance costs associated with *in-situ* and effluent water quality control that are largely negated using cage culture methods may be outweighed by

manual labor costs which are higher in comparison to pond culture of barramundi (Barlow et al., 1996b).

Tank culture systems are constructed from cement, fiberglass or polyethylene fashioned in circular or rectangular (raceway) shapes. Tank system hydrology can be managed as flow-though, recirculated, or a combination of both. Flows through systems have the greatest potential for high nutrient impact on natural ecosystems (Costa-Pierce, 1996). Advances have been made in the area of recirculating systems, namely in-line water quality treatment strategies. Applying these strategies can decrease overall water consumption by enhancing the productive capacity (i.e. longevity) of source water, thereby increasing the number of areas than can be utilized as aquaculture sites (Lucchetti and Gray, 1988).

Efficient recirculation systems can decr
total system volume / day (EIFAC, 1986)
as reported by Black (Black, 2001).
However, highly efficient recirculation
systems require complex and expensive
infrastructure, and access to a dependable
source of energy, as well as skilled
management. Figure 1.1 shows a
commercial recirculation system designed
for efficiency. It is important to note that
most commercial systems, particularly in
developing countries, do not operate
systems which employ all the technologies

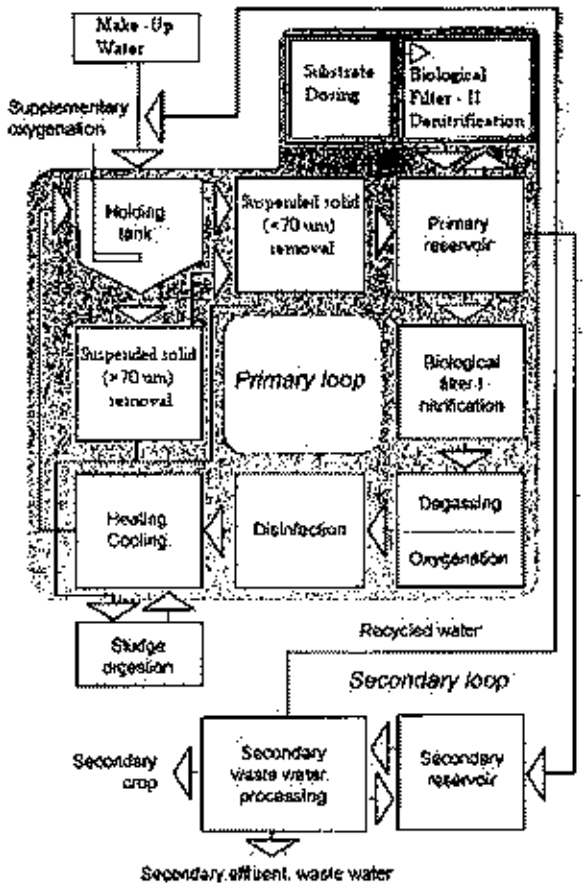


Figure 1.1: Recirculating aquaculture system employing primary, secondary, and tertiary water treatment technologie Figure modified from Black (2001), p. 116.

shown in figure 1.1, most likely resulting in less efficient water usage.

1.1.1 Polyculture

Polyculture is a method of aquaculture which takes advantage of existing custodial niches to increase efficiency (e.g. food production, water quality, and diversity). The niches can be filled with animal and/or plants dependent on availability of organisms that are compatible and culture-able in the system.

Lates calcarifer (Asian sea bass; barramundi) and *Cherax quadricarinatus* (red claw) were the primary and secondary animals chosen for polyculture in systems designed and operated in this experiment. They were chosen because (1) a consumer demand in Australian and foreign markets exists for these commodities, (2) both species are indigenous to Queensland aquatic systems, (3) both have been individually cultured successfully and marketed, and (4) they are particularly suited for the integrated wetland systems detailed in this document. Additionally, *Velesunio ambiguus* (freshwater mussel), *Schoenoplectus validus* (emergent hydrophyte), and *Baumea articulata* (emergent hydrophyte) species were cultured as they performed specific functions in the system that are explained below.

Polyculture of the primary and secondary species (or the five species combined) has not been achieved to date.

1.2 *Lates calcarifer*

Barramundi are finfish indigenous to the Indo-West Pacific region from the Arabian Gulf to China, Taiwan, and Papua New Guinea (Schipp, 1996). At least sixteen genetically distinct strains of Australian *Lates calcarifer* can be found from

the Noosa River (S.E. Queensland) northwest to the Ashburton River (Western Australia). The protandrous and catadromous (Grey, 1987) fish are euryhalent, and inhabit brackish swamps as juveniles, and coastal estuaries - low order rivers as adults that can reach a size of 180 cm and 60 kg (Schipp, 1996). Barramundi can survive water temperatures as low as 15 ° C for short periods in tank culture (Rimmer, 1995), and survival in successful culture operations can be over 90 % (Chaitanawisuti and Piyatiratitivorakul, 1994).

Maximum barramundi food intake occurs at *c.* 29 °C, and decreases with temperature ceasing at *c.* 20 °C (Lobegeiger et al., 2001). A standardized ratio of food assimilation used by the industry is called a feed conversion ratio (FCR). Feed conversion ratios (FCRs) are determined by dividing the amount of dry feed applied to a system by the animal fresh mass gained. Most commercial aquaculture FCRs commonly range between 1.5 - 1.8 : 1, dependent on fish species, food composition, and culture conditions. Fish cultured with Australian produced pellets give FCRs of roughly 1.0-1.2 : 1 under experimental conditions, and 1.6 - 1.8 : 1 under commercial culture (Schwartz and Boyd, 1995, Barlow et al., 1996b).

In Australia, cultured barramundi are fed dry pelleted feed and the typical FCR of commercially cultured barramundi in Queensland is 1.5 - 2.0 : (Barlow et al., 1996a), although fluctuations between 1.85 and 2.35 : 1 had occurred during the period 1997 to 2000 (Lobegeiger et al., 2001). Williams (1996) found barramundi FCRs ranged between 1.1 - 1.4 under laboratory tank conditions where diet composition and temperatures were trialed; under pond conditions barramundi FCR ranged from 1.2 - 1.5. Under variable culture conditions, others report barramundi FCRs of 1.3 - 2.0: 1 (Rimmer, 1995), 0.86 - 1.48 : 1 (Williams et al., 2003), 8.24 -

16.38 : 1 (Chaitanawisuti and Piyatiratitivorakul, 1994), and 1.62 - 1.77 : 1 (Williams et al., 2000). Food conversion rations (FCRs) below 1.0 have been achieved in some experimental *Lates calcarifer* aquaculture systems (Awang, 1987, Schipp, 1996). Published specific growth rates (SGR = $[(\ln W_t - \ln W_0) / t] * 100$) of barramundi range from 0.63 - 0.71 (Chaitanawisuti and Piyatiratitivorakul, 1994), 0.90 - 1.40 SGR (Williams et al., 2003), and 0.66 - 3.60 under varying culture conditions (Williams, 1998).

Computations for a profitable barramundi farm for north Queensland estimate the annual production to be *c.* 3 kg fish production per m^2 of pond surface area (Barlow, 1998). Average culture cost per plate-sized product (300 - 500 g specimen) was reported as between $ 5.00 - 7.50 AUD, with 30-50% of that expenditure allocated to feed cost, and 20-30 % allocated to labor costs(Barlow et al., 1996a, Negroni, 2000). The remainder comprises management, consumables, distribution, maintenance, and other miscellaneous costs.

1.3 *Cherax quadricarinatus*

Cherax quadricarinatus is a nocturnal, euryhalent, omni-detritivore crustacean that is indigenous to Australia (Queensland and Northern Territory), as well as to PNG river systems (Jones, 1990). Red claw adults can reach sizes ≥ 35.0 cm in length and 600 g weight (Farm, 2002b, Farm, 2002a). Jones' (1990) experiments indicated that > 70 % optimal growth can be achieved in a 22.5 to 31.5 °C range (28 °C optimal) of culture water temperature; and that adverse effects on survival and weight were not found at 0, 6, and 12 ppk salinity treatments, lending support to his recommendation that *Cherax quadricarinatus* is "an ideal aquaculture species"(Jones, 1990). Specific growth rates of red claw grown in ponds range

between 0.63 - 1.58 (Jones and Ruscoe, 2000), 1.6 - 1.9 (Pinto and Rouse, 1992), and 1.6 – 2.3 (Austin, 1992). Reported red claw culture FCRs ranged from 1.18 - 1.64 : 1 (Pinto and Rouse, 1996, Rouse and Kahn, 1998), and 2.5 - 8.0 : 1 (Rouse and Kahn, 1998). Survival of over ≥ 90 % is possible in successful systems (Jones, 1990, Meade and Watts, 1995) however survival rates ranging between 28 and 72 % are reported (Jones, 1995, Pinto and Rouse, 1996, Rouse and Kahn, 1998).

1.4 *Velesunio ambiguus*

Velesunio ambiguus are edible freshwater mussels (ENRC, 2002) found in coastal rivers, dams and wetlands of Victoria, South Australia, New South Wales, and Queensland (Conservation, 2002, Williams, 1968). As of 1999 there were eight aquaculture facilities holding species permits in NSW, with three of those practicing commercial production (Fisheries, 1998-99). Value of this organism is approximately $6.00 AUD / kg (Fisheries, 1998-99, Shells, 2002). The larvae of this species are reported to be parasitic on fish (Conservation, 2002) before taking permanent residence in aquatic substrates. The species have been used by indigenous Australians for food and tool construction, especially near the Murray River (Smith and Kershaw, 1979).

1.5 *Schoenoplectus validus*

Schoenoplectus validus (lake club brush, Kapungawha) is a native emergent, euryhaline sedge, the stem of which can reach > 2 m in height and 1.0 cm diameter. Dark green-blue when well nourished, *Schoenoplectus validus* colonizes via rhizomes and grows best in nutrient enriched environments (Commission, 2000). The plant can be maintained in water depths of 60 - 70 cm, can tolerate up to 2.0 ppk

salinity, and can provide a possible human food source as its underground stems are edible (Romanowski, 2000). This species does exhibit a mild seasonal senescence at 23° 24' S latitude (this study), and may exhibit similar senescence in similar, or more temperate climates.

This species has been used in Australian constructed wetlands (Greenway, 1997, Greenway and Woolley, 1999, Tanner, 1996), and Greenway (1999) measured the nitrogen and phosphorus in *Schoenoplectus validus* plants collected from constructed wetlands used to enhance treatment of secondarily treated municipal wastewater. The above-ground and below-ground plant matter concentrations of nitrogen and phosphorus were 14.6 and 2.6 mg g^{-1} (above-ground), and 14.5 and 4.0 mg g^{-1} (below-ground) (Table 1.1).

Greenway (1997) also investigated *Schoenoplectus validus* plants from natural wetlands and free surface constructed wetlands (i.e. the wetland water surface is in direct contact with the atmosphere) used to enhance secondarily treated municipal wastewater. The average total plant concentrations of nitrogen and phosphorus in natural wetland plants were 5.8 and 0.8 mg g^{-1}, respectively, and the average total plant concentrations of nitrogen and phosphorus in constructed wetland plants were 14.3 and 4.0 mg g^{-1} (Table 1.1) Tanner (1996) found nitrogen and phosphorus concentrations in *Schoenoplectus validus* plants collected from a free surface constructed wetland used to treat dairy farm wastewater to be *c.* 21.0 and 3.0 mg g^{-1}, respectively (Table 1.1).

Author	species	system	sample		nitrogen (mg g^{-1})	phosphorus (mg g^{-1})
Tanner (1996)	*S. validus*	dairy farm WW sub-surface wetland	AG		23	2.6
			BG		20	3.4
	B. articulata		AG		20	2.2
			BG		18	5.7
Greenway (1999)	*S. validus*	secondary municipal WW free surface wetland	AG		14.6	2.6
			BG		14.5	4
Greenway (1997)	*S. validus*	secondary municipal WW free surface wetland	WP		14.3	4
	B. articulata		WP		13.1	3.7
	S. validus	natural wetland	WP		5.8	0.8
	B. articulata		WP		12.6	2.4
Adcock (1994)	*B. articulata*	tertiary municipal WW free surface wetland	AG		1.5	11.8
			root		8.4	1.6
			rhizome		6.5	18.8
	B. articulata	natural wetland	AG		4.2	0.3
			root		7.1	0.7
			rhizome		4.9	1

Table 1.1. Nutrient concentration of *Schoenoplectus validus* and *Baumea articulata* from constructed and natural wetlands. AG = above ground biomass; BG = below ground biomass, WP = whole plant biomass, WW = waste water.

1.6 *Baumea articulata*

Baumea articulata (bamboo reed, jointed twig rush) is a native, emergent sedge, the stem of which can reach 2.5 m height and 1.3 cm diameter, and is green. *Baumea articulata* colonizes via rhizomes, and has been described as quick-growing and robust in nutrient limited environments (Romanowski, 2000).

The plant can be maintained in water depths > 1 m, tolerate up to 1.0 ppk salinity, and dry soils for extended periods (Commission, 2000, Romanowski, 2000). Senescence is not as pronounced as for *Schoenoplectus validus* at 23° 24' S latitude

This species also has been used in Australian and New Zealand constructed wetlands (Greenway, 1997, Greenway and Woolley, 1999, Adcock and Ganf, 1994, Tanner, 1996). Greenway (1997) investigated *Baumea articulata* plants from a natural wetland and a constructed wetland used to further enhance secondarily treated municipal wastewater. The average whole plant concentrations of nitrogen and phosphorus in natural wetland plants were 12.6 and 2.4 mg g^{-1}, respectively, and the average whole plant concentrations of nitrogen and phosphorus in constructed wetland plants was 13.1 and 3.7 mg g^{-1} (Table 1.1). Tanner (1996) found nitrogen and phosphorus concentrations in *Baumea articulata* plants collected from a constructed wetland used to enhance dairy farm wastewater were *c.* 19.0 and 4.0 mg g^{-1}, respectively (Table 1.1). Adcock (1994) investigated *Baumea articulata* plants from a constructed wetland used to enhance tertiary treated municipal wastewater and a natural wetland. The average total plant concentrations of nitrogen and phosphorus constructed wetland plants were *c.* 4.5 and 11.0 mg g^{-1}, and in natural wetland plants the average total plant concentrations of nitrogen and phosphorus were 5.1 and 0.6 mg g^{-1}, respectively (Table 1.1).

1.7 Aquaculture water quality control

Water quality control in aquaculture is a major concern for aquaculturists as it directly affects animal production, capital / operating costs, and aquaculture permitting procedures. Chemical mass balance (section 2.10) of undisturbed aquatic ecosystems (e.g. rivers, wetlands, estuaries) is regulated by cyclic/rhythmic internal,

ecologically adjacent, and global biogeochemical / physical processes working to maintain water quality naturally. In recirculating aquaculture systems, accumulation of waste products and by-products are an inevitable result. Practically all wastes generated within aquaculture systems originate from fish feed (directly or indirectly); estimates of 5 -15 % of food introduced is lost directly into the system uneaten (Black, 2001) and roughly 80 % of food digested by fish is excreted (Hopkins, 1989).

1.7.1 Solid wastes

Nutrient-rich aquaculture solids are composites of unconsumed feed, fecal material, and floc of variable size, composition, and settling velocity. Composite fractions can include plankton, soil particles, bacteria, protozoans, fungi, algae, plant debris, metals, and nutrients.

Total solids can be classified by state, size, and chemical/elemental structure, and encompass all materials in solution, excluding gases (Tchobanoglous, 1985). Settleable solids can be separated gravimetrically from the recirculation stream of aquaculture systems using in-line settling basins or pond structures. Common strategies employed to biodegrade and utilize the fish solids captured by settling basins include land application, aerated or anaerobic lagoons, and activated sludge technology. Suspended solids can act as adsorption substrates for bacteria, viruses, protozoans, trace metals, and nutrients; induce fish stress (Redding et al., 1987); facilitate disease outbreaks (Bullock et al., 1994); and physically damage the gill structures of various aquatic organisms, thereby reducing their capacity to efficiently exchange metabolic substrates and by-products, specifically oxygen and ammonium (Stickney, 1994). Gearheart (1992) noted that the smaller the particle size, and the more organic its composition, the more likely it would act as a pollutant. Suspended

solids clog biofilters, and as with settleable solids, can cause oxygen depletion and toxic by-products (e.g. ammonia) during decomposition within the water column. Concentrations of suspended solids in aquaculture water must be minimized in order to maintain a healthy, highly productive fish population.

Fish themselves can remove solids from water; Teltsch (1991) showed that planktivorous fish can remove significant amounts of chlorophyll *a* from culture solution through herbivorous feeding, converting primary production into protein. The feeding strategies of many commonly cultured organisms such as mussels act to remove particulate matter from the water column, and have been reported to change the status of water within a recirculating salmon system from a eutrophic to oligotrophic condition (Soto and Mena, 1999).

1.7.2 Carbon

In aquatic systems, organic carbon is the primary energy source for benthic communities, driving sedimentary biogeochemical processes, and in the form of aquaculture wastes, stimulating benthic microbial respiration (Black, 2001). Carbon dioxide (CO_2) is a metabolic by-product of cellular respiration, as well as carbon substrate for chemoautotrophic bacteria and photosynthetic organisms. Aqueous CO_2 generally does not become a limiting water quality parameter in aquaculture systems, however, as concentrations increase it may asphyxiate fish, reduce biological filter efficiencies, and cause a drop in pH (Grace and Piedrahita, 1994). Control of aqueous CO_2 can be achieved via chemical pH management or gas exchange. In traditional recirculating aquaculture systems it is assumed that allochthonous / autochthonous carbon that is not organically bound or sequestered in sediments is eventually lost within effluents, solid wastes, and/or diffused into the atmosphere.

1.7.3 Nitrogen

Nitrogenous compounds are components of amino acids (protein building blocks within organic molecules) that are vital to the growth of organisms. Ammonia is a gaseous nitrogen configuration that is the primary metabolic by-product of protein digestion in fish (Burrows, 1964), and a decomposition product of the organic matter within wastewater (Ogden, 1994).

Concentrations of total nitrogen in fresh waters range between 0.1 and 2.0 mg/L (Boyd, 1979). When present in its non-ionized form (NH_3) aqueous ammonia is highly toxic to many aquatic species (Lucchetti and Gray, 1988). Short-term exposure to 0.6 mg/L - 2.0 mg/L NH_3 is toxic to most fish (Boyd, 1979), and sub-lethal effects, expressed as reduced growth, organ damage and lowered disease resistance, can occur at concentrations ranging between 0.05 mg/L - 0.3 mg/L (Boyd, 1979, Robinette, 1976, MRTC, na, Burrows, 1964, EIFAC and Commission, 1973, EIFAC, 1986). The occurrence of ionized ammonia (NH_4^+) is dependent on the concentrations of dissolved oxygen and carbon dioxide, as well as temperature and pH (Thurston, 1981 a), and can shift between non-ionized and ionized configurations. The ammonium nitrogen configuration (NH_4^+) is toxicologically harmless to aquatic organisms (Timmons, 1987), and tends to be adsorbed to particulate matter at high pH values (Wetzel, 1975, Mitsch, 1993). Although most other forms of nitrogen are generally non-toxic to fish in an acute sense, they can be assimilated readily by aquatic ecosystems, and are generally thought of as the primary and secondary limiting nutrients in marine and freshwater aquatic systems, respectively (Wood and Hensman, 1989, Stickney, 1994, Costa-Pierce, 1996).

Biological filters (biofilters) are used to control accumulation of toxic

nitrogen compounds within recirculated aquaculture water. Trickle filters are commonly used, although plant-based filters, extended aeration / denitrification systems, and other technologies are available. Complete oxidation (nitrification) of ammonia results in nitrate, a relatively harmless (to fish) nitrogenous compound at concentrations below 50 mg/L (ANZECC and ARMCANZ, 2000). Nitrification is a dual step aerobic reaction that can be mediated by two chemoautotrophic bacteria, *Nitrosomonas* sp. and *Nitrobacter* sp. The bacteria form epiphytic colonies upon attachment substrates such as biofilter media, sediments, aquatic plant roots and stems, and on suspended particulates (i.e. activated sludge processes). The exothermic oxidation reaction permits bacterial assimilation of dissolved CO_2 to form organic molecules (Ogden, 1994). The reaction rate is negligible if oxygen levels are below 2.0 mg/L (Ogden, 1994).

The nitrification process does not remove nitrogen from culture systems. To remove nitrate nitrogen from system, the nitrification process is essentially reversed by facultative anaerobic bacteria such as *Paracoccus denitrificans, Thiobacillus denitrificans,* and *Pseudomonas denitrificans* in a process called denitrification, which is the major removal mechanism of nitrogen from many aquatic systems (Johnston, 1993, Good, 1987). The nitrogen gas end product of the denitrification process can diffuse out of the water column into the atmosphere, effectively removing it from the system. The reaction is temperature dependent and is fuelled by organic carbon made available by decomposition of detrital material, or, in some applications, added in chemical forms such as methanol or raw plant materials. Aquaculture systems can incorporate hydroponics, phytoplankton production, and wetlands to retain and remove nitrogen (and phosphorus) from aquaculture water via harvesting accrued biomass. The plant biomass produced from these methods can be

fed back to fish, consumed by humans and other animals, used as a denitrification carbon source, or in other applications such as compost or biogas operations.

1.7.4 Phosphorus

Phosphorus is used in components of cell membranes, bone, ATP, and a variety of organic and inorganic molecules. As the primary limiting nutrient in freshwater ecosystems (Stickney, 1994, Wood and Hensman, 1989, Costa-Pierce, 1996), more than 90% of phosphorus in fresh water occurs as organic phosphates and as cellular constituents in microorganisms adsorbed to inorganic and dead particulate organic material (Wetzel, 1975). Concentrations of phosphorus in fresh waters range between 10 and 500 µg/L (Boyd, 1979), and trace levels are required to stimulate phytoplankton production (Wetzel, 1975) provided adequate concentrations of other macro and micronutrients are available also. Concentrations of total phosphorus (TP) are used to define the trophic state of lakes: > 50 µg/ L : hypereutrophic, > 20 - 50 µg/ L : eutrophic, 10 - 20 µg/ L : mesotrophic, and < 10 µg/ L : oligotrophic (Carlson, 1977).

Phosphorus has not been reported to cause direct toxic effects in fish, even at the relatively high levels of 5.0 mg/L (Stickney, 1994). In traditional recirculating aquaculture systems, phosphorus accumulation is not considered to be a limiting factor of water quality. It is assumed that allochthonous / autochthonous phosphorus that is not bound organically, or sequestered in sediments, is eventually lost within effluents, or solid wastes. In traditional recirculating aquaculture systems, however, it is evident that input phosphorus not bound in fish mass is eventually lost with effluents and solid wastes.

1.7.5 Oxygen

Dissolved oxygen is often the limiting factor in aquaculture, and is considered the most critical water quality variable in aquaculture (Boyd, 1979, Stickney, 1994, Timmons, 1994). The desirable range of dissolved oxygen (DO) for fish is no less than 5.0 mg/L, with mortality occurring after prolonged exposure to concentrations between 0.3 to 1.0 mg/L (Boyd, 1979). Growth rates and resistance to parasites and disease can be hampered by prolonged exposure to DO concentrations between 1.0 mg/L and 5.0 mg/L (Boyd, 1979, Plumb, 1976).

The rate of oxygen diffusion into and out of water is dependent on water temperature, atmospheric pressure, salinity, oxygen concentration gradients between the aquatic and atmospheric environments, and physical processes such as wind / wave / pump action (Tchobanoglous, 1985). Energy consumptive methods of aeration can provide the oxygen needed to meet the requirements of fish and bacterial respiration in aquaculture systems particularly when high carrying capacities have to be maintained. Aeration can protect also against excessive carbon dioxide, ammonia, methane, and hydrogen sulfide build-up within the water column. The method of aeration used is dependent on the individual aquaculture system, and a number of aeration devices, such as surface paddles and blowers, are available.

1.8 Aquaculture mass balance

The most direct and precise way to ascertain the efficiency of any biological system (production or natural) is by mass balance calculation. Mass balance is an engineering analysis based on the physical law of conservation (i.e. mass is neither created nor destroyed, although it can change form) and is a quantitative description of selected materials that enter, remain within, or exit a system (Tchobanoglous, 1985). The same calculations can be used to determine the potential of aquaculture activities to cause environmental damage. Hall (1990) stated that mass balance analyses form the basis of assessment of eutrophication effects caused by fish farming. Timmons (1994) listed the following step sequence as an approach to mass balance problem solving: 1) define system boundaries, 2) identify materials to be balanced, 3) isolate and identify movement across set boundaries, and 4) identify mass transformations taking place inside the boundaries that affect the mass balance. A powerful aspect of using mass balance calculation as a research tool is that direct comparisons can be made between aquaculture systems, regardless of the system design, culture methods, or animals used in production. Unfortunately, there are no examples in the literature where mass balances are conducted on integrated wetland aquaculture systems, or fish and crayfish polyculture systems (this study represents the first). However, mass balance information for aquaculture per se has been collected.

Schwartz (1994) reported that for each ton of freshwater catfish (*Ictalurus punctatus*) produced, an average of 19.6 kg total nitrogen, 0.87 kg total phosphorus, 39.3 kg biochemical oxygen demand, and 2 302 kg settleable solids were discharged from production effluent and drainage of three ponds located in S.E. United States.

Schwartz estimated that 20.2 % of the carbon, 56.8 % of the nitrogen, and 36.4 % of the phosphorus system inputs were discharged to the environment in the form of escaped fish and effluent / pond draining with the remaining (un-measured) attributed to fish biomass, soil retention, overflow, seepage, or atmospheric diffusion.

Hennessy (1996) estimated fish farm waste loadings from measurements taken at freshwater Atlantic salmon (*Salmo salar L.*) farms located in north west and central Scotland. Total ammonia nitrogen (TAN), total phosphorus (TP), dissolved reactive phosphorus (DRP), and suspended solids (SS) increased as source water passed though the farm sites; with daily waste loadings of 0.11 - 1.71 kg ton fish $^{-1}$ day $^{-1}$, 0.03-0.83 kg ton fish $^{-1}$ day $^{-1}$, 0.00 – 0.08 kg ton fish $^{-1}$ day $^{-1}$, and 1.1 – 20.5 kg ton fish $^{-1}$ day $^{-1}$, respectively. In the same paper, Hennessy (1996) reported values from seven other fish farms, showing that atlantic salmon, brown trout, and rainbow trout cultured in cages, tanks, or ponds, produced anywhere from the following ranges: 0.02 – 0.27 kg ton fish $^{-1}$ day $^{-1}$ TAN, 0.01-.043 kg ton fish $^{-1}$ day $^{-1}$TP, 0.02-0.27 kg ton fish $^{-1}$ day $^{-1}$DRP, and 0.8 - 7.1 kg ton fish $^{-1}$ day $^{-1}$SS.

Costa-Pierce (1996) summarized estimates of total phosphorus (TP) and total nitrogen (TN) in aquaculture effluent in relationship to marine fish production from four cage culture studies. He reported that an average of 0.1 kg to 108.4 kg of nitrogen, and 19.2 kg to 90.2 kg of TP was lost in effluent per ton of fish produced.

Hall (1990) estimated that, in marine cage cultured rainbow trout, 21 - 22 % of total carbon (TC) input was captured in harvested fish and 75 - 78 % (878 - 952 kg t^{-1} fish produced) was lost to the environment in marine cage culture. Total carbon accumulated in the sediment was estimated to be 18 % of the total carbon input. Hall (1992) estimated 27 - 28 % of total nitrogen (TN) input was captured in harvested

fish and 67 - 71 % (95 - 102 kg t^{-1} fish produced) was lost to the environment. Total nitrogen accumulated in the sediment was estimated to be 12 % of the total nitrogen input. Holby and Hall (1991) estimated 17 - 19 % of total phosphorus (TP) input was recaptured in harvested marine fish from the same cages, and 78 - 82 % (19.6 - 22.4 kg t^{-1} fish produced) was lost to the environment. Total phosphorus accumulated in the sediment was estimated to be 59 - 66 % of the TP input.

Handy and Poxton (1993) reviewed published results and reported 52 - 95 % of food nitrogen added to various aquaculture systems was lost via food wastage and fish excretions. Black (2001) noted it was reasonable to expect 30 % of the nitrogen, and 60 % of the phosphorus discharged from temperate cage systems to be in a solid form (e.g. feed and feces).

The restricted number of aquaculture mass balance investigations limits the knowledge base. One basic gap in knowledge is that there are no examples of an integrated aquaculture-wetland system mass balance.

1.9 Aquaculture environmental impacts

Land and aquatic system conversion, loss of biodiversity, water / energy usage, and genetic / pathogen transfers are directly linked impacts of aquaculture activity. Surprisingly, as of 1996, the majority of modern aquaculture facilities did not treat wastewater prior to discharge (Costa-Pierce, 1996). Although regulations regarding wastewater management are becoming stricter in many countries, the level of regulation is variable from location to location. Environmental impacts resulting from aquaculture activities now are under scrutiny by governmental, community and

environmental agencies in developed nations. This issue is the main barrier to potential aquaculturists in that any new aquaculture system must show that it will not cause environmental damage via aqueous waste discharge.

A major threat of aquaculture wastes to receiving aquatic environments is the possibility of hyper-eutrophication: the accelerated ageing of ecosystems due to enrichment with nutrient elements (Welch, 1992). A review of literature by Black (2001) reported the specific impacts associated with increased nutrient content within water bodies: as a) transient or longer term effects upon water column chemistry, b) deoxygenation and enrichment of sediments with organic wastes, c) loss of benthic diversity, and d) the formation of a potential long-term nutrient enrichment source as post-production leaching from benthic deposits occur. The 'symptoms' of accelerated ageing encompass a range of related environmental impacted conditions.

Phillips *et al.* (1991) concluded that the practice of aquaculture would face restrictions in development in many parts of the world due to water resource competition and public concern over detrimental water quality impacts. Stickney (1994) and Timmons (1994) lent support to this prediction by reporting that the quality of aquaculture effluents had become a concern in developing and developed countries with increasing environmental regulation becoming apparent. In addition, Costa-Pierce (1996) noted that the political strength of international and grass-roots environmental organizations have changed dramatically aquaculture policies world-wide. Lastly, the Queensland Department of Primary Industries stated that "unchecked aquaculture development has degraded coastal environments in many parts of south-east Asia; however current licensing and management measures will prevent such degradation in Queensland" (Lobegeiger et al., 2001).

1.10 Wetlands and water quality

Wetlands are among the most important and threatened ecosystems on Earth (Gopal and Junk, 2000). They exist in all climatic zones (excluding the extreme polar regions) from tropical to tundra latitudes, and comprise 6% of Earth's land surface (Gopal and Junk, 2000, Gearheart, 1992). Wetlands provide habitat for vast numbers of aquatic and terrestrial species, and are the primary habitat for many threatened and critically endangered organisms on Earth (Boynton et al., 2002), for example, approximately 10 % of Earth's fishes reside in the Amazon basin alone, 25 % of global vertebrate biodiversity exists in wetlands and inland waters (Stiassny, 1999), and over 95% of United States commercial fish, such as bluefish, sea trout, mullet, striped bass and drum, depend on wetlands for nursery and spawning habitat (Mitsch, 1993). Although only 3% of the landmass of the USA is wetland (Mitsch, 2000), those wetlands are estimated to contain 270 species of birds, over 5000 species of plants, and 190 species of amphibians; and with 45 % of all animals and 26 % of all plants listed as either threatened or endangered either directly or indirectly dependent on those wetlands for survival (Hammer and Bastian, 1989). Niering (1988) reported that 28 % of the plant, 20 % of the mammal, 68 % of the bird, 63 % of the reptile, 75 % of the amphibian, 66 % of the mussel, 48 % of the fish and 38 % of the insect species listed as threatened or endangered within the USA are associated with wetlands. North American freshwater prairie pothole wetlands were estimated to produce 50 - 80 % of North America's game bird species (Batt et al., 1989). Malaysian peat swamps contain approximately 25 % of that country's total plant species (Anderson, 1983). It is logical, therefore, to assume that the wetland benefits identified herein are most likely similar to remaining wetland habitats throughout the world.

Societal benefits afforded by wetlands systems are significant to. Carboniferous period swamps are the origin of oil, coal and other fossil fuels on which current society is heavily reliant, and historically, civilizations have depended on wetlands for fish, wildlife and food production. Examples include: (a) the use of salt marsh vegetation for animal grazing, hay production, and roof thatching in East Africa, Central and South America, Northern Europe, New England, and the British Isles (b) utilizing mangrove swamps for timber, food and tannin production in Indo-Malaysia, (c) timber production and fur trapping in southeastern United States, (d) rice propagation and irrigation in China and Japan, and (e) extraction of fuel energy from peat soils by Russian and Irish communities (Gearheart, 1992). Today the value of wetlands as sources, sinks, and transformers of many chemical, biological and genetic materials is increasingly more evident. Wetlands are among the most productive of Earth's ecosystems (Gopal and Junk, 2000). Given the fact that wetlands are more effective in sequestering atmospheric carbon than many forest ecosystems, their role in absorbing greenhouse gases also is distinct (Gearheart, 1992).

Mitsch and Gosslink (1993) referred to wetlands as "kidneys" when describing the water quality functions they perform as interfaces between Earth's hydrologic, biogeochemical, and atmospheric processes, accepting and transforming organic/inorganic materials of natural and anthropogenic origin. Water quality benefits afforded by wetlands include nutrient cycling and reduction of solids, trace metals, and biochemical oxygen demand (Gearheart, 1992). Sedimentation, physical and chemical adsorption, filtration, and precipitation (Brix, 1993), the interaction of assimilation and chemical transformation processes occurring within and between macrophyte, algal, invertebrate, bacterial, fungal, and actinomycetes

populations, are the primary processes that affect pollutant concentrations in wetland ecosystems (Gearheart, 1992). All of these processes are interrelated and dependent on a number of variables such as wetland hydrology (depth, retention time, velocities, flow pattern), soil condition and constitution, *in-situ* micro-relief variations, influent characteristics, climate, topography, season, management practices (if required), and level of biodiversity. Basic ecological principles state that the more complex the trophic structures of a ecosystem, the greater number of niches and species there will be; and affording resilience, stability, and fluidity to ecosystem structure and function over geologic time (Gearheart, 1994). Given the importance of wetland ecosystems to Earth, the use of natural wetlands to treat wastes (as employed in the past) now generally is considered taboo from an ecological perspective. However, the use of constructed wetlands is a widely accepted, appropriate technology developed in place of natural wetlands to perform many of the same ecological services.

1.10.1 Solids

Solids entering a wetland can remain sequestered in wetland sediments, in plant /microorganism biomass, or be discharged as wetland effluent. Increased hydraulic retention and resistance, afforded by large water surface areas and dense stands of aquatic vegetation, facilitate the removal of solids entering wetland systems by gravitational, adsorptive and absorptive means.

1.10.2 Nitrogen and phosphorus

Nitrogen entering wetlands can (a) volatilize out of the water column in a gaseous configuration (N_2, N_2O, NO_2, NO_3, NH_3), (b) remain in the wetland, held in

the sediments, or as plant /microorganism biomass, (c) be discharged to surface waters within the wetland effluent, or (d) percolate into ground water (Kadlec, 1989, Gearheart, 1992). Nitrogenous gas venting to the atmosphere is the main mechanism by which nitrogen is converted and exported from wetland systems (Good, 1987). The large, surface area within wetlands offers attachment substrates throughout the water column and benthic regions for epiphytic species of nitrogen-converting organisms. Colonization occurs on live plants, detrital matter, and most available surfaces within wetlands (Cattaneo, 1979).

Nitrogen and phosphorus within wetland waters are assimilated readily by resident primary producers such as macrophytes, algae and bacteria. Although there may be a limit to the total amount that can be sequestered at any one time within biological tissue as a wetland reaches its apex of total biomass, the retention of nutrients by this mechanism can be significant as it can take several years for the limits of this process to be reached. The removal of dissolved inorganic phosphorus (DIP) and dissolved inorganic nitrogen (DIN) from the water column to microbial / algal biomass production is considered the primary short-term sink (Gearheart, 1992), however the sink-capacity becomes saturated quickly (Richardson, 1985). The size of a wetland microbial sink, enhanced by luxury consumption activity (i.e. the accumulation of nutrient ions), can change at daily (or shorter) intervals (Tourbier, 1976). There is also a linkage between plant and microbial uptake to other nitrogen (and phosphorus) removal processes (e.g. nitrification in anoxic wetland soils supported by oxygen leakage from plant roots). If plant harvesting is practiced, nutrient removal can be achieved on a continual, annual basis.

Nitrogen can be trapped in sediment as particulate bio-litter accumulated on

the bottom of wetlands during seasonal die-off (Gearheart, 1992, Williams, 1985). The ammonium ion form of nitrogen (NH_4^+) can be trapped physically by clay particles such as vermiculite and smectites residing within wetland soils (Brady, 1984). In addition to the accumulation of organic nitrogen and phosphorus as detrital particles, wetland sediments can retain reactive phosphorus and ammonium (Howard-WIlliams, 1985).

The accumulation of wetland detrital biomass may comprise only a minimal annual nutrient sink to peat production (Nichols, 1983) because a significant portion of nutrients within biomass can be lost back into solution via bacterial/fungal decomposition, fragmentation, leeching and autolysis (Gearheart, 1992). For example, the amount of phosphorus lost from decomposing plant tissue can be 35 - 75% of total phosphorus within its tissues (Richardson, 1985, Nichols, 1983). Seasonal nutrient release back into the water generally occurs disynchronous to the time of year when excess nutrients introduced to aquatic systems are most likely to promote algal blooms and other effects associated with hyper-eutrophication. Spangler (1977) suggested that taking advantage of dysynchronous release processes has the greatest potential for managing nutrient retention in wetlands. In addition, release due to senescence is a relatively controlled process, protecting against large pulses of nutrients entering the system, as might occur in a flood scouring event. Furthermore, co-distributions of microorganisms, root mass and organic matter in the soil surface suggest a transfer of nutrients between live and dead biomass, a key process in the overall bio-regulation of phosphorus and nitrogen retention and transformation in wetlands (Wood and Hensman, 1989).

Sedimentation of plankton, macrophytes, and precipitate / adsorptive forms of

phosphorus can be the major long-term sink of phosphorus in wetlands (Richardson, 1993, Davies, 1993). At low loading rates, wetlands have a capacity to remove much of the phosphorus entering the system, and can continue to achieve removal over several years (Gearheart, 1992). However, as loading rates increase, the efficiency of phosphorus removal declines, as the system becomes saturated (Nichols, 1983). Richardson (1993) noted that then-current data suggested permanent storage of phosphorus in wetlands was generally below 0.5 $g/m^2/yr$.

The atmosphere is not considered a potential phosphorus sink, because the element is generally not thought to exist in a gaseous form (Richardson, 1993). Phosphine is a malodorous, colorless, poisonous gas that occurs on a limited extent (Davies, 1993), however the source of phosphine is not fully understood, as it has not been studied in great detail (Devai, 1988). Its production could be potential but very limited sink for phosphorus from aquatic systems (Gearheart, 1992).

1.11 Constructed wetland - aquaculture integration

Constructed wetlands have proven effective in reducing aquatic pollutants worldwide (Mackney, 1990). Constructed wetlands were defined by Hammer and Bastian (1989) as man-made ecosystems consisting of saturated substrates, emergent and submergent vegetation, animal life, and waters that simulate natural wetland conditions and processes for human benefit. One goal of constructing wetlands for wastewater reclamation projects is to enhance the capacity of constructed wetlands to perform specific and multi-benefit functions as modeled by natural wetland forms.

Black (2001) referred to wetlands, as options for aquaculture water quality

control, as highly productive and significant ecosystems that "may have the potential to process excess nutrients in the food chain without any adverse effects on species assemblage, biological pathways and overall system function", however, "the technology appears to still be in its infancy" with more work required to develop their integration into aquaculture systems. Costa-Pierce (1996) suggested that constructed wetlands and the use of custodial species in aquaculture systems both show great potential as more sustainable methods of aquaculture. Comeau (2001) concluded that the potential exists for constructed wetlands as an ecologically attractive and economical method for treating fish farm effluents; and additionally it could help in obtaining certified "natural" fish production for niche markets (Negroni, 2000).

There are a limited number of works that investigated constructed wetlands – aquaculture integration reported in the literature with the majority of them published since this study began. The remainder of this section outlines those and other examples of wetlands integrated with aquaculture applications, and includes a comprehensive table (located at the end of this section) that can be used to more easily make comparisons between systems (Table 1.2).

Lin (2003) described the performance of a dual-cell pilot scale constructed wetland used to recirculate wastewater from a *Litopenaeus vannamei* (prawn) culture tank located in Taiwan (October – January 2000). Each wetland cell (1.0 m x 5.0 m x 0.8 m dimension) was planted with *Phragmites australis*. The first cell was a free-surface design and the second cell was of sub-surface design positioned physically and hydrologically in tandem. A second prawn culture tank was used as a control. The wetland-facilitated mean percentage of reductions in the concentrations of total

ammonia nitrogen, nitrate, nitrite, phosphate, and suspended solids from the culture tank water of 57 %, 68 %, 90 %, 5.4 % and 71 %, respectively (Table 1.2). The concentrations of suspended solids and nitrates were significantly lower in the culture tank utilizing the wetland cells while mean prawn length, weight, and survival were significantly higher in the wetland integrated culture tank. The author concluded that integrating wetlands into outdoor recirculating *Litopenaeus vannamei* culture systems could remove the major pollutants from the wastewater stream effectively, providing high water quality and a good culture environment, and consequently increasing the growth and survival of the prawn. Hence, applying constructed wetlands could potentially reduce the energy, labor, and capital costs associated with a recirculating aquaculture system.

Lin's research was confined to focusing on the nutrient aspects of nutrient water quality (namely nitrogen and phosphorus configurations). There was no mention of animal culture measurements (e.g. feeding rate, RGR, FCR, water use to fish production ratio, culture density), or other pertinent investigations concerning the integrated system (e.g. evapotranspiration and biodiversity). Additionally, the system described by Lin was not replicated, nor were successive experimental trials run. However, the experiment was run at a pilot scale which gives the work some credibility in 'real world' applications as 'scale effect' is not an issue.

Tilley (2002) described the performance of a 7.7 ha, mesohaline, free-surface (i.e. water surface is exposed to the atmosphere) constructed wetland employed as a recirculating water filter for a full-scale *Litopenaeus vannamei* (prawn) farm located at the Loma Alta Shrimp Aquaculture Facility (Port Mansfield, Texas, USA) over the period of the year 2000. *Typha latifolia* occupied ⅔ of the wetland with ⅓ occupied

with a mixture of *Ruppia maritime*, *Avicennia germinans*, *Chara* spp., *Juncus effuses*, *Pithophora* spp., *Nymphaea odorata*, *Hydrochola carolinensism* , *Sesbania drummondii*, and *Borrichia frutescens*. Wetland hydraulic residence time was approximately 24 hours, and, during recirculation, the wetland reduced total phosphorus and total suspended solids by 31 % and 65 %, respectively. Although ammonia levels increased over the wetland hydraulic gradients, and nitrate remained stable, their concentrations remained below 0.4 mg L^{-1} and 2.0 mg L^{-1} in the water column, respectively (Table 1.2). The author concluded that constructed wetlands could perform satisfactorily as recirculation filters in large-scale prawn culture applications by reducing the impact of effluent on adjacent water bodies, using source water more efficiently, and providing habitat in the way of a healthy and productive brackish wetland.

Tilley's approach to his research, like Lin's (2003) approach, was confined to aspects of water quality and also lacked replication. However, Tilley's work did take place on a large scale system over a relatively lengthy production trial (365 days), lending credibility to his work.

Comeau (2001) published a paper regarding the removal of phosphorus and suspended solids from trout farm effluent located in Province of Quebec (Canada). Two subsurface flow wetlands (20 m x 8.5 m x 60 cm) positioned in physical and hydrological tandem were filled with limestone; the first wetland was planted with *Phragmites australis*, and the second was unplanted. Wetland influent total phosphorus concentrations were between 0.03 to 0.61 mg L^{-1} applied at variable hydraulic loadings over 18 months (May 1998 to October 1999); wetland effluents remained below 0.1 mg L^{-1} over the course of the experiment, achieving an overall

removal of 87 % (Table 1.2). Wetland influent suspended solid concentrations were between 7.8 - 65.5 mg L^{-1} while wetland effluents remained below 4.0 mg L^{-1}over the course of the experiment, achieving an overall removal of 94 %.

Comeau's approach to the integrated system was much like the approaches of Lin (2003) and Tilley (2002): the research was focused exclusively on effluent water quality, and was not replicated, neither in time nor in space.

On the Sinu River in northwest Columbia, Gautier (2001) investigated a 120 ha natural mangrove wetland (*Rhizophora mangle* dominant tree / *Acrostichum aureum* dominant fern system) that was exposed to recirculated prawn culture water from a 286 ha boone prawn (*Litopenaeus vannamei*) farm over 12 weeks (November 1997 to February 1998). Estimated recirculation rates of *c.* 66 - 81 % of pumped pond influent achieved hydraulic retention times (HRTs) ranging 2 - 4 days. Suspended solid removal was 95 % however all nutrient concentrations increased in the wetland in this application (except nitrite) due to the intensely rich biodiversity it supported (Table 1.2). It was thought that the guano from thousands of roosting egrets and herons was thought to be the source of the nitrogen and phosphorus increase within the wetlands, while denitrification probably facilitated lowered nitrite concentrations. Further, it was suggested that using natural mangrove wetlands as biofilters was a new approach, and that prawn pond effluents can enhance the biomass production of certain mangrove seedling species, hence preventing erosion as well as stimulating water productivity. It was suggested that the guano nutrient supplementation (i.e. bird excrement nutrients transfer into water) could be recirculated back for use for prawn pond fertilizing. The author also noted that the performance of natural uncontrolled ecosystems could not be assumed on the basis of theoretical

calculations, and that the long term effects of permanent flood conditions on natural mangrove wetland ecology needed to be investigated.

As in the integrated systems reviewed to this point in this section, the research conducted by Gautier (2001) was focused primarily on water quality (specifically wetland effluent nutrient quality) and was not replicated. However, the system investigated was of a commercial scale. Additionally, Gautier (2001) remarked on the immense biodiversity attracted to the natural mangrove wetland that was utilized as a filter and run under elevated nutrient loading. The decision to use the natural wetland for aquacultural purposes, and to promote the use of natural mangrove systems as prawn pond filtration, are highly questionable engagements given the known ecological importance of mangrove ecosystems to the regional and global ecology.

Costa-Pierce (1998) published a study that employed an aquaculture-wetland ecosystem (AWE). The goal of the study was to determine whether tertiary-treated municipal wastewater (Pomona, CA, USA) could be used to simultaneously accomplish aquatic food production and inorganic nitrogen removal. The AWE consisted of three 48,000 m^2 (200 m x 240 m) polyculture ponds, and one bowl-shaped 0.05 ha (500 m^2) surface flow wetland dominated by *Typha*, water hyacinth and *Lemna* species occupying 25 %, 50 % and 25 % coverage, respectively. Specifics on wetland depth and hydraulics were not reported. Polyculture ponds held tilapia (*Oreochromis mossambicus x O. urolepis hornorum*), carp (*Cyprinus carpio*), mosquito fish (*Gambusia affinis*), and red swamp crayfish (*Procambarus clarkii*), while cultivation of water hyacinth (*Eichhornia crassipes*) and Chinese water spinach (*Ipomea aquatica*) was practiced in the pond, and around the edges

(Table 1.2).

Fish were fed dried pellets (containing 32 % protein) at the rate of 1 % body weight (bw) d^{-1}. Standing densities of both tilapia and carp at harvest were 1134.5 kg and 31.2 kg, respectively, which converted to a total fish density of 1 kg fish per 124 m^3 pond volume (0.008 kg fish/m^3). Tilapia were stocked as 21 g fingerlings, and harvested at small plate size (350 - 401 g) after 202 days of culture (May to November 1996). Total FCRs ranged 0.90 - 1.23 : 1, and SGR ranged 1.44 - 1.66 (tilapia) and 0.11 - 0.21 (carp). Tilapia and carp survival ranged 48 - 64 % and 88 - 100 % respectively (low tilapia survival was due to bird predation). Crayfish and mosquito fish were not included in harvested biomass.

Ammonia and nitrate concentrations ranged 6.2 - 11.0 mg/L, and 0.46 - 1.20 mg L^{-1}, respectively, in Pomona tertiary wastewater used during the study. Ponds were filled initially with a 50/50 mix of tap water and wastewater, then each week were flushed with 20 % volume of wastewater maintaining a batch-flow HRT of 5 day ;(nighttime aeration was necessary). In earlier stages of the work, ponds were filled initially with 100 % wastewater, resulting in fish kills caused by pH $\geq$10.0 and toxic levels of un-ionized ammonia (caused by massive blue-green algal blooms, supported by high levels of solar radiation penetrating a nutrient-rich water column).

Labor-intensive, continual harvest of water hyacinth was employed to limit pond coverage to 50%, and with the harvest hyacinth, used as compost, mulch on footpaths and in agricultural areas, and as goose feed. It was estimated that the maximum standing crop of hyacinth in the ponds was 1.4 ton. While four hundred tons of water spinach was estimated to be the standing crop encompassing one pond. In-pond production of water hyacinth removed 90 % of the ammonia and nitrate-N in

wastewater, and the wetland removed an additional 7%. Wetland effluent contained less than 0.4 mg/L ammonia, and no detectable nitrate (Table 1.2). Wetlands were observed as habitat for a variety of threatened birds (notably black crowned night herons, blue and green herons, and rails) that fed upon pond fish, increasing fish mortality rates. The author acknowledged the limitations of the experimental design, noting that due to the lack of controls, the results could only be treated as speculative.

This work of Costa-Pierce (1998) was the most interdisciplinary, integrated, and comprehensive research completed on a commercial scale, integrated wetland – fish polyculture system until this study. The investigation included both animal and plant production, as well as water nutrient water quality, and noted biodiversity (specifically rare birds). Weaknesses of the study included (a) lack of replication, (b) data collection was conducted over only one fish polyculture period, and (c) specifics on hydraulics were not included (e.g. evapotranspiration and water use to fish production ratio).

Sansanayuth (1996) investigated the treatment of aquaculture pond supernatant effluent from a *Macrobrachium rosenbergii* culture pond located in Chanthaburi Province of Thailand (specific date or season not provided). Two subsurface trenches (13 m x 1.2 m x 0.20 m) dug in parallel were lined with plastic and filled with gravel, each having a void volume of 1.2 m^3. One trench (Wetland 1) was planted with mangrove fern (*Acrostchum aureum*) while the other (Wetland 2) was left unplanted. A batch flow hydraulic regime was followed, discharging effluent into the wetlands at 24 hr intervals over 30 days at loading rates producing hydraulic retention times of 72 hours (experiment days 1-9), 48 hours (experiment days 11-18)

and 24 hours (experiment days 19-30). Total wetland removal of total nitrogen (TN) was 48 %, total phosphorus (TP) removal was 31 %, and suspended solids (SS) removal was 84 % (Table 1.2). Wetland 1 removed more TN and TP than did Wetland 2. The systems evapotranspired *c.* 9-14 % of influent water. Differences between trenches were not noted. The author suggested both systems removed TN, TP and SS although the planted system (Wetland 1) reached higher removal standards. This research conducted by Sansanayuth (1996) resembled that of Lin (2003), Tilleys (2002), and Comeau (2001) in that it was not replicated, and focused exclusively on wetland effluent water quality over a 30 day trial of prawn culture.

Schwartz and Boyd (1995) published a study describing the function of two free-surface wetland cells positioned in tandem, parallel to a 2.8 hectare channel catfish (*Ictalurus punctatus*) aquaculture pond located in Greensboro, Alabama, USA. Each cell measured 84.0 m long and 14.0 m wide, the first of which was planted with California bulrush (*Scirpus californicus*) and giant cutgrass (*Zizaniopsis miliacea*); and the second with Halifax maidencane (*Panicum hemitomon*). An influent manifold applied water to four equidistant points across the front section of cell 1 at a hydraulic loading rate (HLR) of 77-91 L/m^2. Cell 2 received effluent from cell 1, and recirculated it back into the aquaculture pond. Experimental hydraulic loading rates that allowed 1, 2, 3, and 4 day hydraulic retention times (HRTs) within the wetland were trialed over six week time intervals, taking place from October 1992 to September 1993.

Weekly water quality samples were taken from the catfish pond influent, cell 1 effluent, and cell 2 effluent. Samples were analyzed for BOD, total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), total Kjeldahl

nitrogen (TKN), total phosphorus (TP), settleable solids (SS - Imhoff cone) total suspended solids (TSS), and volatile suspended solids (VSS).

Wetlands removed substantial amounts of wastewater nutrients during plant growth and dormant stages. Wetland pollutant removal ranged 37-67% (BOD), 1-81% (TAN), 43 - 98% (NO_2-N), 51-75% (NO_3-N), 45-61% (TKN), 49-84 % (TP), 57-100% (SS) 75 - 87% (TSS), and 68 -91% (VSS) (Table 1.2). Concentration of effluent pollutants tended to follow the influent trend. Differences in pollutant concentrations between influent/effluent in cell 1 were greater than that of cell 2, and were attributed to the higher plant density and initial solids removal in cell 1. The 1 d HRT removed more solids and BOD than the 4 d HRT. Increasing HRT over 1 day did not increase removal of NO_2, SS, VSS, and BOD. The 4 d HRT was most efficient in the reduction of TP and NO_3. Greatest removal of TAN was found in the 2 d HRT, but this was unexplained in the study. Both 2 and 4 d HRTs gave 100 % removal of TSS. In all HRT trials, the wetland removed adequate amounts of nutrients from the water during both vegetative and dormant seasons, meeting United States Environmental Protection Authority (USEPA) recommended limits for aquaculture discharge. Evapotranspiration was 5.8 % and 2.8 % influent over growing and dormant periods, respectively.

Five soil core samples were taken at evenly spaced intervals from the wastewater influent point in wetland cell 1 to the effluent point of wetland cell 2; and analysis showed more concentrated CNP in cores taken at the wastewater influent end. The concentration of CNP in the soil samples taken ranged from 41 to 46 % (C), 1.11 to 0.60 % (N), and 0.11 to 0.03 % (P), respectively.

Plants grew "slowly", and Halifax maidencane had not produced a full

canopy by the end of the study. Sixteen transects of post-exposure plant biomass were collected from the wetland cell. Total plant biomass accumulated in the wetlands was approximately 4.0 kg dry weight / m^2. Information regarding catfish culture (i.e. growth, density, disease), water use / production, and pond attributes (e.g. water temperature / pH), as well as issues of biodiversity, were not reported. The authors acknowledged limitations of the experimental design, noting the impossibility of maintaining the same conditions over the four experimental trials in 1993, and that the use of free-surface wetlands with areas 0.7 - 2.7 times that of integrated culture pond areas was not possible for commercial catfish culture due to land constraints in that region, although the use of wetlands for pond drainage and pond overflow treatment was suggested.

The study lacked replication and information pertinent to production efficiency was not collected. However, aspects of water quality, plant production values, and soil core CNP concentrations were included that expanded the research into a more interdisciplinary, integrated systems approach to the work. Additionally, the commercial scale of the system lent credibility to the work as the chance of scale effect was not a limiting factor.

Brummett (1994) cultured Nile tilapia (*Oreochromis niloticus*) and red claw (*Cherax quadricarinatus*) separately and together in 200 m^2 surface area, 1.0 m deep ponds in South Carolina, USA (April - October 1993). The objectives of the research were to determine the impacts of the polyculture on growth rate and total production of animals as well as reproduction of tilapia. The author described the ponds used as supporting *Typha, Potomogeton*, and *Ranunculus* that covered 30 - 50 % of pond surfaces (the plants were cut at ground level and re-grown over the culture

period). Unpublished previous year data (unpublished) was reported to suggest that plants and decaying vegetation in the pond supported satisfactory crayfish growth rates at the stocking rates used in this study. In most instances, purpose built commercial aquaculture ponds do not include such plants as they can hinder culture management. The pond characteristics described by Brummett were suggestive of unintentional constructed wetlands that developed.

Stocking density treatments of 54 g m^{-2} (1 individual m^{-2}) monocultured fish, 54 g m^{-2} fish plus 5-12 g m^{-2} (2.5 individual m^{-2}) polycultured crayfish, and 5-12 g m^{-2} monocultured crayfish were each trialed in triplicate. Animal feeding rates of 2.5 - 7.5 % bw d^{-1}over the trial were observed using 32 % protein, floating, catfish pellets; additionally, dry alfalfa was applied to the ponds at a rate of 250 kg ha^{-1} every fortnight specifically for red claw forage (Table 1.2). Fish harvest took place after 100 days of culture. Fish weight and spawning were reduced in the presence of crayfish. Individual fish weights ranged 185 - 207 g, with maximum pond culture densities were 163 - 175 g m^{-2} (0.85 - 0.88 individuals m^{-2}) and survival rates of between 82 and 85 %. Growth rate of fish was not investigated. Crayfish harvest took place after 170 days culture. Neither crayfish individual weights (54 - 56 g) nor growth rates (0.036 - 0.044 g d^{-1}) varied significantly between treatments, and culture densities of 50 - 59 g m^{-2} (0.9 - 1.1 individuals m^{-2}) were achieved. Survival rates (of between 36 - 45 %) were attributed to visually verified predation pressure by alligators, egrets, frogs, herons, raccoons and snakes. FCRs were determined using fish and crayfish biomass combined, and pelleted only food inputs, and showed that FCRs were significantly better in fish monoculture (1.38 : 1), than in fish polyculture (1.82 : 1) or crayfish monoculture (8.7:1 : 1).

The authors concluded that crayfish disrupted tilapia feeding and spawning behavior as indicated by lower fish weight and reproduction in the polyculture treatment, although only the fish were affected adversely by the competition in that system. Polyculture of the organisms in that system was feasible if crayfish were the primary species. The possibility exists to use lower crayfish densities to minimize antagonistic behavior inducing reduced fish size and growth rate, but it was not investigated.

This research undertaken by Brummet (1994) focused on replicated animal culture measurements (e.g. feeding rate, RGR, FCR, culture density) trialed which makes it the strongest data set of animal growth with integrated wetland systems in the literature. Of additional interest is that this research was the only research that polycultured fin-fish with *Cherax quadricarinatus* crayfish. Brummet (1994) also noted a variety of wetland biodiversity in the vertebrate phylum, and what was unique in that the wetlands non-intentionally evolved from fish ponds. Brummet's research showed a high level of technology integration (e.g. fin-fish culture, crayfish culture, and wetlands), however there was complete lack of water quality and hydrological data collected.

In summary, there has been a number of independent reports of successful wetland-aquaculture integrations within recent years. Barring to a degree the research conducted by Costa-Pierce (1998), the primary weaknesses shared by such research reported to date includes the failure of investigators to gain a full-system integration perspective through the collection of data on all aspects of individual culture components and the integrated system. The investigations on wetland / aquaculture integrated systems needed to not only include animal culture

measurements (i.e. feeding rate, RGR, FCR, water use to fish production ratio, culture density), but also plant measurements (i.e. RGR and biomass), water quality measurements (i.e. *in situ* and/or effluent nutrient or electrochemical data), and well as measures of biodiversity, or any other aspect important to the function of that system to be taken at close enough intervals throughout the study in order to be able to assess the impacts and benefits of integrating components both on the individual component and on the system as a whole.

Additionally, with the exception of the work by Brummett (1994), such studies are of limited value since investigators failed to incorporate replication of both physical design, and/or by successive production trials. As indicated, only Brummet's 1994 work produced specific information concerning the combined function of integrated systems (i.e. that red claw disrupted the spawning and feeding behavior of Nile tilapia). Although that information was not wetland specific, the study was physically wetland inclusive, and the information is applicable to polyculturists that practice similar fish/crayfish polyculture – wetland integrated approaches.

Making comparisons between wetland integrated systems is complicated because each system had several characteristics making them unique from one another (e.g. animal and plant species cultured, fresh water or marine water, system design, topography, etc.). A prime example is that many of the constructed wetlands described in this section were those of subsurface flow design (i.e. the water surface below wetland substrate), therefore quite different than the free surface constructed wetlands (i.e. water surface is exposed to the atmosphere) in other systems. However, such contrasts and comparisons between systems was necessary to

evaluate the current status constructed wetland integrated aquaculture systems research, and establish the first review of such works.

Gaps in the integrated - wetland aquaculture limited body of knowledge certainly exist. For instance, until this present research there had not yet been a description of a wetland – aquaculture integrated systems that gave definitive recommendations on both animal and plant aspects of the systems. Additionally, excluding he experimental design of Brummet (1994), replicated research was not apparent among published research.

Furthermore, in the one study where wetlands served as an ecosystem supporting red claw growth in a custodial niche venue (Brummett, 1994), the culture was physically mixed with herbivorous fish and fed pelleted food directly. Therefore, it was not known if red claw could be cultured in wetlands, or if their growth and survival could be based exclusively on the fish wastes that are (as a byproduct of water quality enhancement) converted to natural food sources in the wetlands, as well as on the physical shelter the wetland plants would provide. If red claw can be grow and can reproduce under those conditions, the value of that species to aquaculturists, polyculturists, and integrated systems would be validated.

author	animal cultured	system type	days studied	stocking weight (g)	harvest weight (g)	growth rate (SGR)	feeding rate % bw d^{-1}	FCR
Brummet (1994)	*Oreochromis niloticus*	plant invaded pond	100	54	185 - 207	-	2.5 - 7.5	1.38 - 8.7 : 1
	Cherax quadricarinatus	plant invaded pond	170	2 -5	54 - 56	0.034-0.037	2.5 - 7.5	-
Schwartz and Boyd (1995)	*Ictalurus punctatus*	culture pond + wetland recirculation	-	-	-	-	-	-
Costa-Pierce (1998) [1]	*Oreochromis mossambicus* x *O. urolepis hornorum*	culture pond + wetland recirculation	202	21	362 - 404	1.44 - 1.66	1.0	0.9 - 2.3 : 1
	Cyprinus carpio	culture pond + wetland recirculation	202	456	611 - 703	0.11 - .021	1.0	0.9 - 2.3 : 2
Comeau (2001)	freshwater trout	treatment wetland	540	-	-	-	-	-
Lin (2003)	*Litopenaeus vannamei*	culture pond + wetland recirculation	80	-	-	-	-	-
Tilley (2002)	*Litopenaeus vannamei*	culture pond + wetland recirculation	365	-	-	-	-	-
Sansanayuth (1996)	*Macrobrachium rosenbergii*	treatment wetland	30	-	-	-	-	-
Gautier (2001)	*Litopenaeus vannamei*	natural wetland	-	-	-	-	-	-
	Table 1.2 continued on next page							

Table 1.2. Integrated wetland – aquaculture systems reported in the recent literature, delineated by author, animal cultured, system type, days studied, animal stocking weight (g), animal harvest weight (g), growth rate (SGR), feeding rate, and food conversion ratio. Stocking and harvest weights (g) are for individual animals. The symbol (-) represents no investigation completed. The symbol (-) represents no data reported.

[1] *Gambusia affinis* and *Procambarus clarkii* were included also in this system, although performance values were not reported.

Table 1.2 (continued)

author	maxi-mum culture density (g m^2)	temp ° C	survival %	*in-situ* water quality (mg L^{-1})	effluent water quality (mg L^{-1})	wetland pollutant removal (%)	ET % of water inputs	ratio of pond area to wetlan d area (m^2)	diversity
Brummet (1994)	163 - 175	-	82 - 85	-	-	-	-	-	alligators, egrets frogs, herons, raccoons and snakes
	50 - 59		36 - 45						
Schwartz and Boyd (1995)	-	-	-	-	-	TKN 45-61 TAN 1-81 NO_2 43 -98 NO_3 51-75 TP 49-84 SS 57-100 TSS 75 - 87 VSS 68 -91	2.8 - 5.8	0.7 - 2.7	-
Costa-Pierce (1998)	8	20	48 - 64	-	NH_3 0.4 NO_3 nil (wetland)	NH_3 90 (pond) NO_3 90 (pond) NH_3 7 (wetland) NO_3 7 (wetland)	-	-	threatened birds
	8	20	88 - 100	-	-	-	-	-	-
Comeau (2001)	-	-	-	-	TP < 0.1 SS < 4.0	TP 87	-	-	-
Lin (2003)	-	-	-	-	TAN 0.09 NO_3 0.12 NO_2 0.004 FRP 7.99 SS 10	TAN 57, NO_3 68, NO_2 90, FRP 5.4 TSS 1	-	-	-
Tilley (2002)	-	-	-	NH_3 < 0.4 NO_3 < 2.0	NO_3 < 2.0 NH_3 < 3.0 FRP < 0.2 TP < 0.7 SS < 150	TP 31 SS 65	-	-	-
Sansanayuth (1996)	-	-	-	TN 4.4 - 4.8 TP 0.15 - 0.17 SS 44 - 55 Temp °C 23-28	TN 1.7 - 2.4, TP 0.04 - 0.11 SS 5 - 11 Temp °C 24-27	TN 48 TP 31 SS 84	-	-	-
Gautier (2001)	-	-	-	-	TSS 5 - 12 FRP 0.2 - 0.8 TAN 0.1- 0.5 NO_3 0.4-1.2 Tmp °C 27-29 pH 7.0-7.5 DO nil - 1.0-	SS 95	-	-	1000s of wetland birds

Table 1.2 (continued). Integrated wetland – aquaculture systems reported in the recent literature, delineated by author, maximum animal culture density (g m^2), water temperature (° C), survival (%), *in-situ* water quality (mg L^{-1}), effluent water quality (mg L^{-1}), wetland pollutant removal (%), evapotranspiration (ET) (% of water inputs), ratio of pond area to wetland area (m^2), and diversity. ET = evapotranspiration.

1.12 Fish and crayfish polyculture

There has been considerable research in fish polyculture in many forms with many species. This section reviews publications describing *Cherax quadricarinatus* polycultured in pond systems with Nile tilapia or other bottom feeding fish, especially if integration techniques resembled techniques developed in this experiment. A comprehensive table located at the end of this section can be used to more easily make comparisons between systems (Table 1.3).

Karplus (2001) was the first to polyculture red claw in recirculating tank systems at what is considered high densities (previous descriptions of red claw polyculture with fish were limited to shallow earthen ponds and relatively low animal culture densities (Rouse and Kahn, 1998, Brummett and Alon, 1994)). The aim of Karplus' study (august to December 2001) was to determine the efficacy of red claw rearing in an polyculture with tilapia; and specifically to determine the effects of crayfish density on tilapia performance and crayfish yield, as well as the need for crayfish shelters in that system.

Twelve circular tanks of 5.5 m^3 volume were connected with a biological filter such that tank effluent recirculated through the filter and back to the tanks at 18 L min^{-1}. Treatments imposed were tilapia at a density of 33 individuals m^{-3} (660-693 g m^{-3}), polycultured with red claw at densities of 20 individuals m^{-2} (140 g m^{-2}) and 10 individuals m^{-2} (70 g m^{-2}) with shelters, and 10 individuals m^{-2} without shelters. Shelters were made of 25 cm long x 7.0 cm wide, black ethylene pipe; and tanks were covered with nets. Fish were fed 35 % protein pellets and at rates suggested by Zoah (1986) in high density tilapia feeding tables. Crayfish were fed at 4 % bw d^{-1} using 40 % protein sinking pellets 4 days a week, and a carrot, wheat, and corn

mixture 3 days a week. Average water quality remained suitable for culture with dissolved oxygen at 81 % saturation, pH at 7.7 - 8.2, ammonium at < 0.06 mgL^{-1}, and water temperature 20 - 30 °C.

Contrary to what Brummett (1994) concluded (see section 1.11), red claw in this system did not impact on the production of tilapia: on the contrary, the fish benefited by out-competing the crayfish in feed foraging. However, it should be mentioned that although the natural wetland plant stands in Brummett's system would have afforded some degree of shelter, Brummett (1994) did not use polyethylene pipe shelters.

Fish raised with crayfish by Karplus (2001) had significantly higher growth rates than mono-cultured fish, however differences in neither fish fresh weight at stocking (20.4 - 20.7 g) and harvest (271.4 - 301.1 g) , nor total crop yield, nor FCR, were significantly different between experimental treatments. Experimental fish growth rate (1.9 - 2.1 g d^{-1}), FCR (1.67 - 1.71), and survival (92 - 95 %) were found to be indicative of successfully polycultured fish crops. Fish culture densities of 5.3 - 5.4 kg m^{-3} were achieved.

The treatment that included crayfish populations without access to shelter was discontinued due to only 2.7 - 2.9 % survival after 14 weeks (a significant effect). There was no significant difference in survival between low and high density, sheltered crayfish treatments. Amongst sheltered treatments, individual crayfish growth rates and mean weight were higher for the low density (low individual number) crayfish treatment, although total crop biomass was significantly greater in the higher culture density treatment. Overall, mean crayfish weight at harvest ranged 31- 36 g, growth rate was 0.18 - 0.21 g d^{-1}, and survival was 57 - 60

%. Crayfish culture densities 188 - 403 g m^{-2} were achieved.

Overall, with respect to fish – crayfish interaction in polyculture, Karplus (2001) showed, importantly, that when crayfish shelters were employed, polyculture of red claw in intensive tilapia culture units was achievable. Karplus in doing so, successfully developed a new integration technique that combined red claw production into high density tank culture of tilapia. One considerable weakness of the research is that of application: the fish and crayfish cultured did not reach marketable size and thus a gap in knowledge exists in terms of commercial feasibility. Furthermore, an experimental design weakness was that a monoculture red claw treatment was not included in the experiment, hence the impact of fish on the red claw growth was unknown (although previously investigated by Rouse and Kahn, 1998), albeit under different conditions.

Rouse and Kahn (1998) polycultured red claw and Nile tilapia in nine 0.02 ha ponds (earthen bottoms and concrete walls) in Alabama, USA to determine the impact of the fish on red claw growth. Red claw were stocked at 14 g m^2 (two individuals m^{-2}); tilapia were cultured at two densities 19 g m^{-2} (1 individual m^{-2}), and 9.5 g m^{-2} (0.5 individuals m^{-2}); and control ponds consisted of mono-cultured red claw. Animal feeding rates with 32 % protein sinking pellets ranged 3 - 5 % bw d^{-1} over the trial with dry alfalfa applied to the ponds at a rate of 250 kg ha^{-1} every fortnight (specifically for red claw forage). There was no mention of shelters provided in the ponds.

Water temperature ranged 23 - 33 ° C, and dissolved oxygen (DO) ranged from 2.4 - 12.5 mg L^{-1}. Un-ionized ammonia in monoculture ponds averaged 0.02 mg L^{-1} while that of polyculture ponds' averaged 0.06 mg L^{-1}, and nitrates remained below

0.05 mg L^{-1} in both.

Harvest took place after 135 days of culture. Red claw individual weights ranged 48 - 76 g, and pond culture densities were 18 - 35 g m^{-2}. Growth rates ranged 0.3 - 0.5 g d^{-1} and were significantly higher in the monoculture treatment than in the polyculture treatment. Survival rates of 19 - 24 % were attributed to poor health upon arrival from Australia. Tilapia individual weights ranged 403 - 444 g, and pond culture densities were between 0.19 - 0.33 kg m^{-2}. FCRs ranging 8.0:1 (in monoculture ponds) to 2.5:1 (in polyculture ponds) resulted.

In the presence of adequate food supply, significantly poorer growth and lesser yields were achieved in the polyculture ponds. The authors suggested the docile and skittish nature of the red claw was a characteristic which deemed the species unsuitable for polyculture with bottom feeding carp and tilapia under free-forage conditions at typical culture densities.

Importantly, the Rouse and Kahn (1998) investigation of low density fish and crayfish interactions in pond polyculture found that the growth of red claw was reduced when polycultured with tilapia, apparently due mainly to a physiological mismatch in character between the fish and the crayfish (living under conditions of no shelters). Significantly, the animals were grown to marketable sizes in this study, which is important when evaluating the economical efficacy of the research results.

Karplus (1995) investigated polyculture of red claw with tilapia hybrid *Oreochromis niloticus* x *O. aureus*, with common carp (*Cyprinus carpio*), and with silver carp (*Hypophthalmichthys molitrix*). Tilapia and carp were stocked at densities of 0.75 m^{-2} and 1.0 per m^{-2}, and cultured for 92 days in four 400 m^2 earthen

ponds located at the Dor Fish and Aquaculture Research Station. Fish were fed 25 % protein commercial carp pellets six days a week. Water temperature ranged 21 to 31 °C over the experiment.

Crayfish growth ranged 0.31 - 0.35 g d^{-1}, producing harvest specimens of 33.2 g average weight, and survival ranged from only 22.3 to 27 % with high levels of physical damage apparent, as only 25 % of harvested crayfish were intact. Tilapia, silver carp, and common carp growth rates and mean harvest weights were 1.6 g d^{-1} at 172.4 g, 7.3 g d^{-1} at 364.4 g, and 4.7 g d^{-1} at 560.9 g, respectively. African catfish (*Claris gariepinus*) of 150 – 250 g and freshwater crabs (*Potamon potamios*) of *c.* 38 g also were harvested from the ponds, and were thought to be responsible for the high level of red claw damage and mortality. It was noted that the red claw did have ample crevices to use as shelter among the stony banks of the ponds.

The author suggested that addition of crayfish did not impact on fish growth in the ponds, but noted negative impact of the fish upon the crayfish. The lack of monoculture control is a noticeable deficit in experimental design. Nevertheless, useful information concerning the function of integrated systems as it relates to fish and crayfish interaction in pond polyculture was generated. The success of red claw culture is reduced when polycultured with the mixture of fin-fish (and given stony banks as shelter) due at least in part to physical damage. That the animals were grown to marketable sizes lends confidence to the technique.

In summarizing this section, investigations of fish and crayfish polyculture to date has focused heavily on production values and on the interaction of Nile tilapia and red claw under variable density and sheltered/non-sheltered conditions. One commonality of studies is that red claw were fed directly, and care was taken to

make sure red claw has access to food. Instead of having to forage; in no instance did red claw fulfill a preferred role in polyculture systems - that of the custodial niche dweller. As noted in section 1.12, it was unknown until this study whether red claw could be cultured within a system without compensatory feeding or management.

With respect to the integrated and polyculture systems presented in both sections 1.12 and 1.13, there was yet to be successful design, management and comprehensive evaluation of a recirculating tank polyculture system that integrated free surface constructed wetlands, moderate to high density fin-fish culture (specifically barramundi), and red claw production. The work conducted for this thesis aimed, in part, to bridge the gaps in knowledge outlined in section 1.8, 1.12 and 1.13, and further aimed to make additional integrations and linkages to terrestrial primary production (such as floral culture), and biodiversity creation and support, with quantitative evaluation of these. Finally, beyond generating tested results and specific information concerning the function of integrated systems in terms of animal, plant, and water subjects, this study also endeavored to develop an interactive layman's tool in respect to integrated systems management (presented and discussed in Chapter V – Dynamic system modeling).

Author	animal	system type	days studied	stocking weight (g)	harvest weight (g)	growth rate g d^{-1}
Karplus (2001)	*Oreochromis niloticus*	tank + biofilter	133	20 - 21	271 - 301	1.9 - 2.1
	Cherax quadricarinatus (males)	tank + biofilter	98	7	31-36	.018 - .021
Rouse & Kahn (1998).	*Oreochromis mossambicus x O. urolepis hornorum*	pond	135	19	403-444	-
	Cherax quadricarinatus	pond	135	7	48-76	0.3-0.5
Karplus (1995)	*Cherax quadricarinatus*	pond	92	3	33	0.31 - .035
	Oreochromis niloticus x O. aureus	pond	92	23	172	1.6
	Cyprinus carpio	pond	92	131	561	4.7
	Hypophthalmichthys molitrix	pond	92	364	1037	7.3

Table 1.3 (continued)

author	animal cultured	feeding rate % bw d^{-1}	FCR	maximum culture density (g m^2)	temp °C	survival %	*in-situ* water quality (mg L^{-1}) unless otherwise stated
Karplus (2001)	*Oreochromis niloticus*	Zoah (1986)	1.67 - 1.71	5292 - 5420	20-30	92 - 95	DO 81 % sat, pH 7.7 - 8.2 NH_4 0.06
	Cherax quadricarinatus (males)	4	-	187 - 403	20-30	57-60	DO 81 % sat, pH 7.7 - 8.2 NH_4 0.06
Rouse & Kahn (1998)	*Oreochromis mossambicus x O. urolepis hornorum*	3-5	2.5-8.1	194-326	23-33	84-90	NH_3 0.02 - 0.06 NO_3 < 0.05
	Cherax quadricarinatus	3-5 + 250 kg ha^{-1} every 14 d	2.5-8.0	18-35	23-34	19-24	DO 2.4 - 12.5
Karplus (1995)	*Cherax quadricarinatus*	-	-	-	21 - 31	22.3 – 27	-
	Oreochromis niloticus x *O. aureus*	-	-	-	21 - 31	90	-
	Cyprinus carpio	-	-	-	21 - 31	94	-
	Hypophthalmichthys molitrix	-	-	-	21 - 31	80	-

Table 1.3. Fish and crayfish reported in the literature, delineated by author, animal cultured, system type, days of activity, initial animal stocking weight (per fish), animal harvest weight (g), growth rate, feeding rate % bw d^{-1}, food conversion ratio, maximum culture density (g m^2), temperature °C, survival %, and *in-situ* water quality (mg L^{-1}).

2.0 Experiment - Freshwater polyculture of *Lates calcarifer* and *Cherax quadricarinatus* integrated with constructed wetlands

2.1 Objectives, performance goals, hypotheses

2.1.1 Objectives

The objectives of this experiment were (a) to integrate barramundi and red claw culture (in polyculture) and to produce and quantify successive animal crops of commercial relevance; (b) to integrate polyculture (see (a)) with constructed wetlands to test and quantify their application as in-line, discharge-water quality control systems, as well as secondary aquaculture medium; (c) to characterize the system as a whole in terms of a CNP mass balance, plant and animal growth efficiencies, aspects of biodiversity, and water quality and usage; and (d) to compare / contrast the performance of two emergent macrophyte species chosen for use in the polishing constructed wetland components of the system.

The anticipated outcome was a pilot model system of sustainable methods, for commercial aquaculturists, which resulted in an ecologically and economically efficient linkage of fish and crayfish production, water quality control, biodiversity creation, and environmental support.

2.1.2 Performance goals

Performance Goals: can be used when designing fish production systems and constructed wetlands for water quality control (Costapierce, 1998, Boyd, 1979, Schwartz and Boyd, 1995). The five goals of this experiment were as follows

Goal 1: Mean fish growth rates will be $\geq$ 1.5 % body weight d^{-1}.

Goal 2: Fish feed conversion ratios will be $\leq$ 1.5 : 1.0 (Barlow 1996, section 1.2)

Goal 3: Water usage will be $\leq$ 1.0 m^3 per 1.0 kg fish harvested.

Goal 4: The integrated system will discharge through its polishing wetland components $\leq$ 10 % of the total nitrogen, phosphorus and carbon that was measured entering as fish feed.

Goal 5: The concentration of total nitrogen (TN), oxidized nitrogen (NO_x), ammonium (NH_4^+), total phosphorus (TP), filterable reactive phosphorus (FRP), and suspended solids (SS) leaving the system as wastewater will not exceed water quality trigger levels suggested by the Australian and New Zealand Environment and Conservation Council (ANZECC) and the Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ).

With respect to goal 5, ANCECC / ARMCANZ (2000) guidelines were chosen because of their history in development. The Australian and New Zealand Ministry of the Environment commissioned ANZECC/ ARMCANZ to develop Australian and New Zealand Guidelines for Fresh and Marine Water Quality. The primary objective of the guidelines were "to provide an authoritative guide for setting

water quality objectives required to sustain current, or likely future, environmental values for natural and semi-natural water resources in Australia and New Zealand" (Hickey, 2003). The trigger values are the concentrations of the key performance indicators (e.g. nutrient pollutants), below which there is a low risk that adverse biological effects will occur; the triggers are not "magic numbers" and are to be used in conjunction with professional judgement (ANZECC and ARMCANZ, 2000).

2.1.3 Hypothesis

Macrophyte Comparison: Null Hypothesis

There will be no difference between *Baumea articulata* and *Schoenoplectus validus* polishing wetland treatments in regard to the following wetland performance characteristics:

- Total carbon, total nitrogen, and total phosphorus sequestered in plant, algae, and soil component;
- Total nitrogen, oxidized nitrogen, ammonia, total phosphorus, filterable reactive phosphorus (FRP), and suspended solids discharged in the wastewater effluent;
- Terminal water output distribution and *in-situ* electrochemical water quality;
- Emergent plant biomass;
- Plant canopy surface area and light penetration;

- Plant photosynthetic rate;
- Benthic invertebrate composition and density; and
- Frog density.

Macrophyte Comparison: Alternate Hypothesis

There will be a difference between *Baumea articulata* and *Schoenoplectus validus* polishing wetland treatments with regard to the same characteristics as outlined in the null hypothesis (see above).

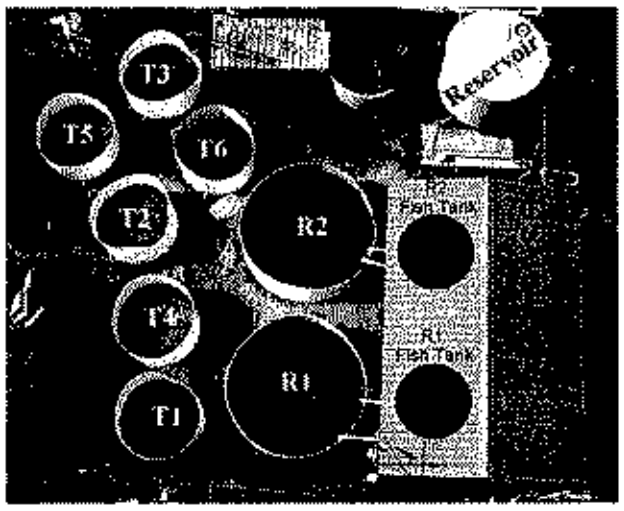
2.2 Experimental design

After an initial six month period of system design, site difficulties and industrial changes beyond the author's control necessitated subsequent system re-design and project relocation to Central Queensland University (see Appendix A - 34).

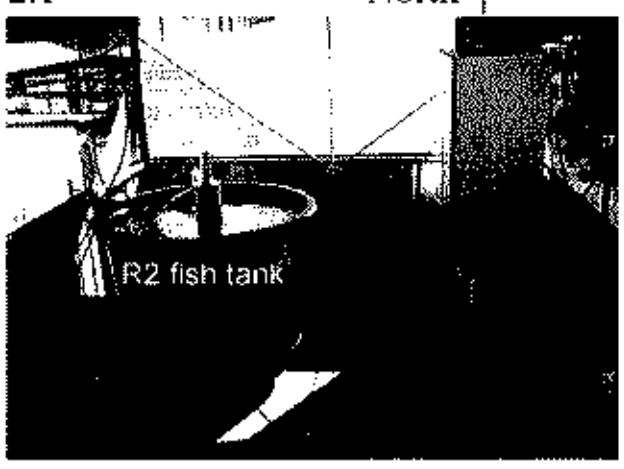
2.2.1 Infrastructure and system design

The system was located on a 200 m^2 plot opened to the project at Central Queensland University, city of Rockhampton, Queensland, Australia (23° 24' S / 150° 30' E). The integrated system consisted of duplicate polyculture - constructed wetland modules (R1 and R2), and six constructed wetlands that were used as water

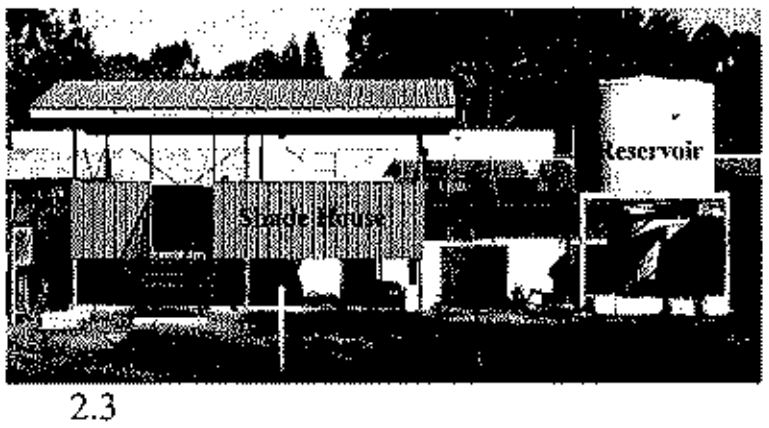
treatment or polishing ecosystems (T1 -T6) (Plates 2.1-2.3). Wetlands were integrated into the aquaculture system researched in this study; and they were used to control recirculated and effluent water quality, provide a culture medium, and increase local biodiversity.



2.1 North ↑



2.2 North ↑



2.3

North ⟶

Plates 2.1 - 2.3: Aerial view of study site (2.1), inside shade house (2.2), and an East perspective of integrated system (2.3).

Components R1 and R2 each consisted of a fish culture tank (fish tanks R1 and R2) hydrologically linked to a corresponding wetland (wetlands R1 and R2). Wetlands R1 - R2 were referred to collectively as culture wetlands, since they hosted *in-situ Cherax quadricarinatus*, and support *ex-situ Lates calcarifer* aquaculture. Wetlands T1-T6 were referred to collectively as polishing wetlands because they collected and treated R1-R2 wastewater. The system followed a batch-flow hydrology (explained in detail within section 2.4.11); and the polishing wetland water (once received from the culture wetlands) was not recirculated back into the culture wetlands or fish tanks, but discharged into a wastewater catchment basin. Recirculation of water only took place between fish tanks (R1 and R2) and their corresponding culture wetlands (R1 and R2).

Wetlands R1- R2 were planted with a mixture of *Schoenoplectus validus* and *Baumea* articulata (details explained in section 2.3.2). Wetlands T1-T3 were planted with *Schoenoplectus validus* and T4-T6 were planted with *Baumea articulata*, providing two monoculture wetland experimental treatments, trialled in triplicate. *Schoenoplectus validus* and *Baumea articulata* were chosen because 1) they are well suited to survive in continuous flood conditions; 2) the species commonly inhabit wetland systems of Central Queensland; 3) the species had yet to be trialed in comparison within free surface design wastewater wetlands 'free surface' meaning the wetland water surface is in direct contact with the atmosphere), and in wetlands treating low strength wetland / polyculture wastewater, and 4) there were limited data in the literature regarding performance characteristics of each species.

2.2.1 Infrastructure and system design

A recirculating design was conceived that required much less water thank the system employed), yet fulfilled the hydraulic needs of a pilot scale system. This design employed two circular fish culture tanks linked to eight constructed wetlands (plate 1). Land preparation consisted of remnant structure removal prior to constructing subterranean pipe drainage, a wastewater catchment basin, and leveling with ground fill. Two galvanized platforms (2.5 m x 2.5 m x 2.0 m and 6.0 m x 3.0 m x 1.0 m) were constructed on 30 cm thick cement slabs: one slab supported a 6000 L, aerated fresh water reservoir that received city tap water (heated by a 3000 watt electrical element), and the other supported two 1000 L polyethylene fish culture tanks and associated aquaculture gear (Plates 2.1 – 2.3). Eighteen millimeter, weather-proof, plywood sheet flooring was fastened to the upper surface of the platforms. A galvanized frame shade house outfitted with a corrugated aluminum sheet metal roof and walls (lower half corrugated aluminum; upper half with 90 % shade cloth) protected the aquaculture equipment from rain and sunlight (Plates 2.1 - 2.3).

The aeration mainline previously installed at the CQU aquaculture unit was linked perpendicularly to a 50 mm PVC air conduit trenched parallel to the western edge of the reservoir and shade house cement footing. Three 50 mm columns were tapped vertically into the trenched conduit, positioned 1.0 m in from both ends of the shade house, to provide aeration for the freshwater reservoir, fish tanks and the recirculation wetlands. Wetland airlines R1 and R2 were secured to the pump influent line along the bottom of the wetland and two 50 mm holes were punched in

the line at the centre of each wetland. Airlines routed to R1 and R2 fish tanks were attached to the bottom of the custom armature that housed the system heating element, which hung vertically into each fish tank; and four 2 mm holes were punched near the airline end plug. All aeration lines were subjected to continual pressure changes each time valves or tank depths were changed in the both the university and the experimental systems, resulting in variations in the amount of oxygen entering the systems. An electrical terminal was installed to provide power for the experiment and surrounding applications.

Constructed wetlands R1 and R2 were fashioned by cutting off the tops of two 3.7 m diameter poly tanks, to form 1.2 m tall, wetland tanks. T1 - T6 were fashioned by cutting off the tops of six 2.14 m diameter poly tanks, to form 1.2 m tall, wetland shells. All wetlands were filled with commercially available, washed, 10 mm quartz river rock to a 20.0 cm depth, and fitted with depth gauges and drainage valves constructed of vertically placed, perforated PVC covered with substrate. Rainwater was free to enter all the wetlands and recorded by a Campbell Scientific CR10x rainfall data logger positioned 4 meters SW of wetland T1 on the roof of a storage shed *c.* 3 m in height.

The fish tanks were positioned in the shade house, 10 cm above the level of the wetlands located outside. The fish tanks utilized gravity drainage from the bottom via vertically mounted PVC manifolds that directed effluents into the corresponding free-surface constructed wetlands (R1 or R2). Water within R1 and R2 was drawn from the far edge (furthest point from influent point) and recirculated back to the fish tanks in a clock-wise direction (the natural tendency of drainage). The ONGA BR-465 swimming pool pumps used to drive the recirculation housed

internal PVC basket traps with a mesh size of 2.5 cm. The construction phase was completed approximately nine months after project initiation (Jan 15, 2001).

2.3 Methods – organizations involved and system set up

In this study the resources of three main organizations were involved in sample collection and analyses. Central Queensland University - Centre for Environmental Management (CEM) & School of Chemical and Biomedical Sciences carried out the water nitrogen and phosphorus analyses, and invertebrate identification as follows. Total nitrogen was determined by flow injection analyses (FIA) using LACHAT (FIA manufacturer) technical method 31-107-04-1A following persulfate digestion. Oxidized nitrogen was determined by FIA following LACHAT technical method 31-107-04-1A. Ammonium was quantified by the Quick Chem method 31-107-06-1-A (indophenol blue). Total phosphorus was determined by FIA using LACHAT technical method 31-115-01-3B following persulfate digestion. Filterable reactive phosphorus analyses were completed by FIA analyses following LACHAT technical method 31-115-01-3B. The author completed suspended solids analyses on water samples using methods outlined in section 2540 - D of APHA (APHA, 1998). The CEM completed invertebrate identification on wetland benthic samples that were collected by the author.

University of Queensland: School of Land and Food Sciences - Analytical Services (FSAS) carried out animal / plant / soil carbon, nitrogen, and phosphorus (CNP) analyses as follows. Total carbon (Keerven et al., 2000) and nitrogen

(Matejovic, 1995) analyses were completed using a LECO CNS-2000 combustion Analyzer at 1100 °C. Phosphorus analyses were completed by nitric acid digestion (Tecator digester block) followed by analyses with an Inductively Coupled Plasma - Optical Emission System (ICP-OES) against external calibration. Additionally, Trial 2 water analyses were completed by FSAS using methods as defined in the previous paragraph. Total nitrogen was determined by flow injection analyses (FIA) using LACHAT technical method 31-107-04-1A following persulfate digestion. Oxidized nitrogen was determined by FIA following LACHAT technical method 31-107-04-1A. Ammonium was quantified by the Quick Chem method 31-107-06-1-A (indophenol blue). Total phosphorus was determined by FIA using LACHAT technical method 31-115-01-3B, following persulfate digestion. Filterable reactive phosphorus analyses were completed by FIA analyses following LACHAT technical method 31-115-01-3B. The author completed suspended solids analyses using methods outlined in section 2540 - D of APHA (APHA, 1998).

Central Queensland University - Plant Sciences Group (PSG): The author completed all other procedures, methods, and analyses, the details of which are presented in the appropriate sub-sections of this dissertation.

The remaining sub-sections of 2.3 encompass system priming methods, that is, the methods followed up to, and including, the stocking of the systems with animals.

2.3.1 Wetland substrate

Wetland substrate consisted of 1 cm diameter, quartz rocks (river rock)

obtained commercially from Betascapes (Rockhampton, Queensland, Australia). During the filling of wetlands with the quartz, five 15 cm radius by 20 cm height (0.014 m^3) substrate samples were taken at regular intervals (approximately one every hour of filling wetlands) to determine the concentration of total carbon, nitrogen, and phosphorus affiliated with the river rocks. Each sample was washed in RO water using a 0.5 / 0.25 cm stainless steel wire and mesh 'chips' basket set into 30 L stainless steel collection / evaporation trays. All wash water was collected and evaporated in collection trays inside an Axyos microdigital drying oven, leaving all residual substrates (e.g. interstitial soil particles and dust) for dry weight analyses and mortar and pestle hand pulverization. After pulverization, sub-samples were secured in 25 ml plastic vials and sent to FSAS for total CNP analyses. Multipliers were required to scale-up the core sub-sample substrate volume to reflect the total wetland substrate volume.

2.3.2 Wetland planting

Schoenoplectus validus and *Baumea articulata* wetland plants were introduced to enhance water quality and biodiversity within the wetlands. Sand cultured plants were obtained commercially from Dragonfly Aquatics, Forrest, New South Wales, Australia (aquatic macrophyte nursery). Wetlands T1-T3 were planted with *Schoenoplectus validus*, and wetlands T4-T6, and R1-R2 were planted with *Baumea articulata*, on February 4th, 2001. Immediately and prior to wetland planting, the fresh weight of each plant was recorded. Each wetland (R1-R2 and T1-T6) was planted with equal numbers and total fresh weight of plants by placing nine individual plants equilaterally on 70.0 cm centers (i.e. each plant was 70.0 cm from

the other), in 10.0 cm water depth. By mid-March, it was observed that R1-R2 *Baumea articulata* were not growing fast enough to reach the estimated 50 cm minimum shoot height required to survive in R1-R2 during culture operations. Therefore, fifteen locally obtained *Schoenoplectus validus* plants were planted into each R1 and R2 wetland (on 50.0 cm centers) to ensure that an adequate amount of plant biomass (possessing taller shoots) was established at the commencement of fish culture activity. *Schoenoplectus validus* plants used in supplemental planting were removed from a riverine wetland located between the towns of Yeppoon and Emu Park (Queensland, Australia) on March 24th. Supplemental planting of R1-R2 took place on March 26, 2001, and as for earlier plants, all were weighed prior to planting but, additionally, great care was taken to wash soil and debris from plants taken from the riverine wetlands. Constructed wetlands were left to establish prior to commencing aquaculture activity on July 4th, 2001.

2.3.3 Wetland planting - fresh weight analyses

Dead shoot / root materials were removed from the experimental plants. Plant roots then were washed with reverse osmosis water (RO) to remove residual soil, gently compressed between dry absorbent paper for five seconds on upper and lower sides before weighing fresh with a Sartorius LG 12000s digital scale, and placed into corresponding wetlands.

Sub-sampling of plants entering the wetlands was undertaken to estimate plant dry weight, shoot surface area, and total CNP concentrations (partitioned as shoot and root fractions) existing at the time of planting as described below.

2.3.4 Plant sub-sampling - fresh and dry weight analyses

Fifteen plants were sub-sampled from each species (including supplementary planting stock) and treated in the same fashion as the wetland planting samples. The samples were then separated into shoot and root sections, and the shoots were re-weighed. The difference between total plant and shoot mass was recorded as root fresh weight.

Shoot and root sub-samples were dried at 70 ° C for 72 hr and weighed (g) with a Sartorius LG 12000s digital scale immediately upon removal from the drying oven. Sub-sample dry weight percentages were calculated by dividing sub-sample dry weights by corresponding fresh weights (x 100), with the resulting data used to estimate the amount of dry weight plant matter entering the wetlands during planting activity by multiplying the calculated sub-sample percentage dry weight by the fresh weight of plants entering the wetlands.

2.3.5 Plant sub-sampling - wetland canopy surface area

Shoot surface area was measured between fresh and dry mass analyses. Fresh shoots were cut into sections before compression onto the scanning surface of a HP Scanjet 4C digital scanner. The resulting images were analyzed with a Dt-scan software program, which measured the surface area (mm^2) of the silhouette image produced. To compensate for the one-sided image, the value measured was multiplied by a factor of two. Sub-sample shoot surface area was divided by the sub-

sample total plant dry weight to calculate the shoot surface area produced per gram of total plant dry weight. The resulting values were used as multipliers to estimate the total shoot surface area of the wetland canopy, accomplished by applying the multiplier to the estimated total plant dry weight entering each wetland (Section 2.3.4).

2.3.6 Plant sub-sampling - carbon, nitrogen and phosphorus (CNP) analyses

Dried below and above ground samples were combined into five below-ground and five above-ground samples for each plant species. Each sample contained the dry matter from 3 plants. Samples were cut into pieces with coarse dissection scissors, and then ground with an IKA Labortechnik - MFC model hammer mill, followed by pulverization for 24 hr in glass jars using a Crompton Parkinson 1.5 automated roller and alloy grinders. Samples were secured in plastic sample vials and sent to FSAS for analysis. The total amount of CNP entering the wetlands via plant matter was determined by multiplying the dry weight of plants entering the wetlands by the concentration (mg g^{-1}) of CNP in corresponding sub-sample analyses.

2.3.7 *Lates calcarifer*, *Cherax quadricarinatus*, and *Velesunio ambiguus* stocking and sub-sampling

Fingerling *Lates calcarifer* used in the experiment were obtained commercially from Kuranda Fish Farm (Queensland, Australia) on four separate

occasions for use in three grow-out trials over the course of the study. Ethical clearance to grow the fish was granted from the CQU Ethics Committee prior to receiving the fish. Trial 1 fish stocking began on July 4, 2001 with harvest on November 3, 2001: each tank was stocked with 40 fish (40 fish m^{-2}). Trial 2 fish stocking began on June 21, 2002 with harvest on November 15: each tank was stocked with 15 fish (15 fish m^{-2}). Trial 3 fish stocking began on February 27, 2003: each tank was stocked with 115 fish (115 fish m^{-2}). However, one hundred percent mortality in R2 within the first week of production due to pump priming failure forced a second fish stocking to occur in R2 on March 27, 2003. As a consequence, R1 fish harvest took place on July 18, 2003 and R2 harvest on August 21, 2003 in trial 3.

Immediately upon arrival, fish were acclimated for two weeks in a 1.5 m^3 isolation tank located at the CQU aquaculture unit. The fish were treated with a 15.0 g L^{-1} total dissolved solids (TDS) salt bath for six hours after the first week of isolation to cleanse them any external parasites. Fish were transferred into each culture tank in the experimental system after anaesthetization with 17.0 ppm *Aquis* (clove oil) for 15 minutes, weighing (g) individually with a Sartorius LG 12000s digital scale, and measuring (mm) total length with a flexible measuring tape.

Fish were not fed for 24 hr prior to any sampling or sub-sampling. Fish sub-sampling was undertaken to estimate dry mass and CNP mass of the fish entering the system at the start of each grow-out trial. In trial 1 four individuals chosen randomly were not anaesthetized, but transferred to ice slurry for 20 minutes. After weight and length measurements were taken, they were immediately freeze dried for 5 days at -100 ° C, placed into desiccators for 3 days post-freezing, then weighed (g) with a

Sartorius LG 12000s digital scale to determine dry weight (g). Sub-samples then were sent to FSAS for CNP analyses. In trial 2 six sub-samples were collected, put on ice, and processed in the same fashion. Trial 3 fish were not included in the CNP mass balance.

Juvenile *Cherax quadricarinatus* used in the experiment were obtained commercially from Winnmore Crayfish Farm, Rockhampton, Queensland, Australia. All introduced crayfish were treated with 5.0 mg L^{-1} TDS salt bath for 15 min to cleanse them of any external parasites. Crayfish were weighed with a Sartorius LG 12000s digital scale, and measured in millimeters (from tip of head to bottom of the telson,) using a flexible measuring tape prior to transfer into their respective culture wetlands.

In trial 1, ten crayfish were stocked into each culture wetland on July 7, 2001 and harvested on January 31, 2002 (the progeny of which survived in the wetlands un-aided to become the subjects of trial 2). Trial two crayfish (surviving trial 1 progeny) were harvested on December 14, 2002. Trial 2 progeny were killed in the wetlands during the dry-out phase where not enough water was available for the red claw to survive; the dry-out was required to complete the wetland CNP mass balance. In trial three, 20 crayfish were stocked in each culture wetland on March 3, 2003. Crayfish of similar size to those residing in the wetlands at that time were added on April 23, 2003 to R1 (7 additional crayfish) and R2 (6 additional crayfish) because the dense vegetation proved difficult when trapping adequate numbers of specimens for monthly evaluations. The trial three crayfish harvest occurred on August 24, 2003.

Trapping was accomplished using standard nylon mesh (5 mm) mud crab

traps (50 cm x 30 cm x 30 cm) purchased from Barra Jacks, Rockhampton, Queensland, Australia. The traps were baited with 2-3 pellets of 10 mm size fish feed (see section 2.4.1 for more information on feed) held in 25 mm plastic vials perforated with 1 mm holes, tied inside the traps; the traps were set in the water at dusk and pulled up every hour for three hours, or until most of the crayfish were caught. End of trial crayfish harvests were achieved through night spearing (in addition to trapping) using a custom triple barb spear, with the aid of torches, and complete wetland draw-down (in trials 2 and 3 only) to ensure a total adult population determination.

Crayfish sub-sampling prior to the commencement of each trial was necessary to estimate dry mass, and CNP mass entering the culture tanks as crayfish. In trial 1, seven individual crayfish selected at random were kept in 50 L buckets containing aerated, dechlorinated tap water for 24 hrs prior to freeze drying for 3 days at -100 ° C. They then were desiccated in a Gelman Scientific 15 L desiccator for a further 3 days, and re-weighed to determine % dry mass. Three of the seven dried sub-samples were sent to FSAS for CNP analyses. Trial 3 crayfish were not included in the CNP mass balance.

Juvenile *Velesunio ambiguus* (freshwater mussel) were stocked in the culture wetland systems to enhance water quality via the 'filter feeding' strategy of this organism. *Velesunio ambiguus* used in the culture wetlands were obtained from McCormick's fish farm (Balina, New South Wales, Australia). Immediately upon arrival, 40 mussels were placed in four 50 L buckets containing aerated, dechlorinated tap water for 72 hours. Mussels then were weighed (g) with a Sartorius LG 12000s digital scale, and surface-cleansed with 15 ppk salt water to remove any

external parasites or commensals. The mussels were stocked into R1 and R2 wetlands on July 5, 2001. Initially, the mussels were kept in mesh net bags strung across wetlands in 20 cm of water, positioned 50 cm from the fish tank effluent point however many mussels slipped though the mesh bags and filamentous algae proliferated over the mesh space. Instead, the mussels were released to free range in the wetlands.

Mussel sub-sampling was necessary to estimate dry mass and CNP mass entering the culture wetlands as mussel. A Sartorius LG 12000s digital scale was used to measure the total weight (g) and the fresh meat weight (g) of 5 randomly selected mussels. Each meat sample was freeze dried for 3 days at -100 ° C, placed into a Gelman Scientific 15 L desiccator for 3 further days, and re-weighed to determine dry weights. One meat sample was taken randomly from the initial 5 with the four remaining meat samples combined randomly into pairs. Each pair represented one of two samples that were sent to FSAS for CNP analyses.

2.3.8 Feed sub-sample and analyses

All food that entered the system was measured at intervals that coincided with fish weight analyses. Three 5 g samples were taken from the food bags directly prior to their use at 50 day intervals during each polyculture trial. Each sample was weighed (g), freeze dried for 3 days at -100° C, desiccated for 3 further days, then re-weighed with a Sartorius LG 12000s digital scale to determine dry mass (g). Two of the five samples were pulverized first with a mortar and pestle, and then further pulverized for 24 hr in glass jars using an automated roller and alloy grinders. Samples then were secured in 25 ml plastic vials and sent to FSAS for nutrient analyses. Bulk portions of fish feed were kept frozen until use.

2.4 System performance measurements

The sub-sections of 2.4 outline the methods followed while animal polyculture was taking place.

2.4.1 *Lates calcarifer* growth, feeding, and survival measurements

Fish were fasted for 24 h prior to data collection to ensure accurate body weight determination. Individual fish fresh weight (g) and total length (mm) measurements were taken from R1-R2 fish tanks on a monthly basis. Fish were transferred by dip-net from culture tanks to green-plastic trash bins containing aerated reservoir water with 17 ppm *Aquis* anesthetic. After 15 minutes, the fish were hand-transferred individually to a Sartorius LG 12000s digital scale for individual weight determination, and then measured (total length, mm) using a flexible measuring tape before transfer back to their respective culture tanks. The following calculations were used to evaluate culture efficiency:

Specific growth rate (SGR is relative growth rate (RGR) multiplied by 100):

$$SGR = RGR \times 100$$

where $RGR = [(\ln W_t - \ln W_0) / t]$

W_t = fish fresh weight (g) at harvest;

W_0 = fish fresh weight (g) at stocking;

t = time.

Survival: % survival = (initial # fish - # dead fish / initial # of fish) 100.

Feeding and feeding conversion ratios

Automatic feeders were not used (as is the normal practice by barramundi farmers), so as to allow observations of the activity of the fish prior to hand-feeding to satiation (see Rimmer, 1995). In this study, fish were hand-fed twice daily to satiation using commercially available Ridley Fish Culture pellets (10 % fat - 45 % protein). Fish feeding rates were calculated, retrospectively, as the mean percentage of food fed to the fish in relationship to their weight on a daily basis (% body weight (bw) d^{-1}) over the experiment. The first step in the calculation uses a linear growth model, which determines mean daily fish growth (% bw d^{-1}) by extrapolating (over a set number of days) the mean individual weight of fish at stocking to the mean individual weight of the same fish at harvest. The number of days set in the extrapolation matched the number of days between fish stocking and harvest measurements. The start and end sections of a 147 day culture period are shown in Table 2.1. Initially, the X value was estimated, with accuracy increasing through tailoring the number until running the equation matched the real data.

$A\,[1.0 + (X / 100)] + A = A_2 \rightarrow A_2\,[\,1.0 + (X / 100)] + A_2 = A_3 \rightarrow A_3\,[\,1.0 + (X / 100)] + A_3 = A_4 \rightarrow$ run for number of days between measurements....(e.g. A_{147})

where X – Percent % body weight d^{-1};

A - Stocking: Mean fish weight (g);

$A_{2.3.4..}$ Mean fish weight during culture period (g).

The linear function used to determine fish growth then was built upon to calculate the mean food consumption per fish (expressed as % body weight d^{-1}) over

the culture period. Mean food consumption rate was calculated by first determining the amount of feed applied per fish:

f/t = d (average daily amount of feed consumed by individual fish)

where f = total amount of fish feed used per tank (g) / # of fish in tank at harvest;

t = fish culture time in days.

The value d was used in conjunction with extrapolation technique to determine fish growth rate. Table 2.2 shows the start and end sections of a 147 day culture period expanded to include food consumption. The d value (11.67) is located in the lower right hand block.

Fish growth extrapolation	12.42 x 1.02589945	12.7468 x 1.02589945	table break	493.6137 x 1.02589945	506.39797 x 1.02589945	519.5134 x 1.02589945
Time →	day 1	day 2		day 145	day 146	day 147
Initial fish weight (g) 12.42	Growing fish (g) → 12.74680	13.07694		506.39797	519.5134	Harvest fish weight (g) 532.97

Table 2.1. Example for a 147 day culture period applying mean fish weights at stocking (12.43 g) and harvest (532.97 g). The growth measurement equals 2.59 % body weight d^{-1}, and can be found in the fish growth extrapolation row as the number 1.0259945.

Fish growth extrapolation	12.42 x 1.02589945	12.7468 x 1.02589945	table break	493.6137 x 1.02589945	506.39797 x 1.02589945	519.5134 x 1.02589945
Fish food consumption extrapolation	12.7468 x 0.0214924	13.07694 x 0.0214924		506.39797x 0.0214924	519.5134 x 0.0214924	532.97x 0.0214924
Time →	day 1	day 2		day 145	day 146	day 147
Initial fish weight (g) 12.42	growing fish (g) → 12.74680	13.07694		506.39797	519.5134	Harvest fish weight (g) 532.97
Accumulated food usage (g)	eaten food (g) → 0.273959	0.281055		10.88371	11.16559	Feed used per fish (g) 11.45

Table 2.2. Example for a 147 day culture period, expanded to include fish food consumption. The total amount of food used per fish (11.45 g) is applied at day 147; the mean food consumption measurement equals 2.15 % body weight d^{-1}, and can be found in the fish food consumption extrapolation row as the number 0.0214924.

A food conversion ratio (FCR) was used to determine food assimilation efficiency, and determined using the following calculation:

FCR* = feed applied / (fish harvest biomass - stocking biomass)

* Units are fresh weight grams, and reported as a ratio (i.e. feed applied to fish growth attained).

2.4.2 *Cherax quadricarinatus* growth and survival measurements

Individual crayfish weight and length measurements were collected monthly from R1-R2. Crayfish were first collected using a combination of non-baited pipe traps, and standard mud crab mesh traps baited with 2-3 pellets of 10 mm fish feed housed in a 25 mm perforated vial suspended inside the trap. Collected crayfish were put into 50 L plastic buckets containing aerated culture water, and then hand-transferred individually to a Sartorius LG 12000s digital scale for fresh biomass (g) determination. A flexible measuring tape was used to measure crayfish (from tip of head to bottom of the telson, mm) before being placed back into their respective

wetlands.

Crayfish growth was calculated using SGR following methods described in section 2.4.1. Food conversion ratios calculation was not possible as the amount of food provided to the crayfish by wetland primary production and associated fish culture wastes could not be quantified directly.

2.4.3 *Velesunio ambiguus* growth and survival measurements

Individual mussel weight measurements were taken on December 20, 2001, after 175 days of wetland habitation. Mussels were collected with nets from wetlands R1 - R2 and held in 50 L plastic buckets containing aerated culture water prior to weighing, after which they were returned into their corresponding wetlands. Mussel growth was calculated using SGR methods described in section 2.4.1. Collection attempts in 2002 were not successful, and it was concluded the mussels had died out.

2.4.4 *Litoria fallax* density

A visual method was used to count individual mature (i.e. tailless) frogs in the wetlands on November 19, 2001 and 2002 at 12:00 pm. Frogs in tadpole stages were not counted. In the larger, more densely vegetated wetlands (R1-R2), red synthetic rope sections were strung across wetlands perpendicular to each other, transecting in the centre, thus dividing the wetlands into quadrants. John Clarke, Resource Ranger at Queensland Parks and Wildlife Service, Yeppoon, Queensland, Australia, made positive species identification. Larval frogs (tadpoles) restricted to

the water column were not counted.

2.4.5 Benthic invertebrate sampling and analyses

Benthic core sample sites were selected using a random number generator programmed with the numbers 1-8 to identify two of eight 45° wedge-shaped, semi-quadrant sections of each wetland. Once drawn, the numbers were not returned to the drawing pool so as to avoid planning of duplicate or multiple samplings at the same spot. If sites designated for invertebrate analysis were found to be within a plant clump, the nearest location where a sample could be collected without significant plant damage within the semi-hemisphere was substituted. Each sample was taken 20 cm from the wetland edge within the randomly chosen wetland sections.

Benthic macro-invertebrate sampling was performed on November 21, 2001 and November 21, 2002 using a 0.027 m^3 volume (30 cm x 30 cm) net (0.3 x 0.9 mm mesh) driven into the wetland substrate to a 20 cm depth, scooping out wetland substrate samples. Substrate samples were placed into rectangular sorting trays and the collection net was rinsed over the tray to remove adhered organisms. Samples were searched systematically for 15 minutes each in shallow wash-water, and visible specimens collected by forceps were fixed in a 50 ml glass bottle (one bottle per wetland) with 70 % ethyl alcohol (ETOH). The substrate samples were returned to their respective wetland sample points after completion of the visual removal of visible specimens.

The same random number procedure was used to choose the sample sites for

the post-experiment wetland benthic substrate CNP cores (section 2.4.15) directly prior to selecting the benthic invertebrate sample locations as described in this section and further; the sites selected for CNP cores were not included in the benthic invertebrate sampling site random number drawing pool.

2.4.6 Wetland Plant Biomass

The rate of increase in wetland plant biomass over 703 days was expressed as relative growth rate (RGR):

$$RGR = [(\ln W_t - \ln W_0) / t]$$

where W_t = plant dry weight (g) at harvest;

W_0 = plant estimated dry weight (g) at stocking;

t = time.

2.4.7 Wetland canopy surface area

Shoot surface area was measured on harvest sub-samples using the methods described in 2.3.5 with the following changes. The multipliers were generated by dividing the sub-sample shoot surface area by the sub-sample shoot dry weight to calculate the surface area (mm^2) produced per gram of shoot dry weight. The multipliers then were used to estimate the total shoot surface area of the wetland canopy by applying them to the total shoot dry weight recovered from the wetlands at harvest.

The rate of canopy surface area expansion in wetland plant biomass over 703 days was expressed as relative surface area expansion rate (RSR):

$$RSR = [(\ln W_t - \ln W_0) / t]$$

where W_t = wetland canopy surface area at harvest (cm^2);

W_0 = wetland canopy surface area at stocking (cm^2);

t = time.

2.4.8 Wetland canopy light penetration

Light penetration was measured monthly with an AccuPAR Ceptometer (light interceptor). Measurements were taken first above, then below, the wetland canopies (at water level) at equal points around the perimeter of each wetland (T1-T6 : 17 points per wetland, R1-R2 : 32 points per wetland). Each single measurement consisted of 20 electronic readings taken 0.5 sec apart, including a sensor function to compensate for plant movement in wind. Photosynthetically active radiation measurements taken at water-level were divided by corresponding above-canopy photosynthetically active radiation measurements, to provide estimates of the incoming photosynthetically active radiation penetrating to the water surface, or inversely, to provide estimates of the amount of photosynthetically active radiation intercepted by wetland canopies.

The rate of decline in light penetration through the wetland canopy to the water surface over 551 days was expressed as relative light penetration decrease rate (RLDR):

where RLDR = $[(\ln W_t - \ln W_0) / t]$

W_t = % wetland canopy light penetration at last sample date;

W_0 = % wetland canopy light penetration at first sample date;

t = time.

Note that the inverse of canopy light penetration equals canopy light interception; and hence, the relative rate of light penetration decreases, and light interception increases, are the same value.

2.4.9 Rate of net photosynthesis

Wetland plant photosynthetic rate (i.e. the rate of fixation of atmospheric carbon dioxide), expressed as μmol m^{-2} sec^{-1} was measured monthly using an Analytical Device Company - Model LCA 4 infrared gas analyzer (IRGA). Measurements were taken 2.5 cm from the shoot tip of five shoots chosen randomly in each wetland. The shoot tip area was selected because a small diameter area was necessary for use in the IRGA sampling chamber. Because the sample chambers assumed a 6.25 cm^2 leaf area (trial 1) or an 11.35 cm^2 leaf area (trial 2), mathematical corrections were applied. The actual shoot surface area within the IRGA chamber was determined by excising twelve shoot tips 2.5 cm in length from 10 to 15 randomly chosen shoots in each wetland set (R1-R2, T1-T3, and T4-T6). Excisions were taken 5.0 cm from the shoot tip (the shoot areas sampled in the IRGA chamber), and the scanning method of surface area analysis, as outlined in section 2.3.5, was used to measure the surface area of the shoot excisions. The chamber area assumptions (6.25 or 11.3 cm^2) were divided by the excision surface areas, and the

means were used as correction multipliers (multiplied by the IRGA result) to determine the corrected carbon dioxide fixation rate of the shoot portion measured.

2.4.10 Water quality - wetland *in-situ* electrochemistry measurements

A TPS 90-FL water quality meter was used to take fortnightly measurements of dissolved oxygen, pH, total dissolved solids, and water temperature from wetland water columns. Measurements were taken at both 10:00 am and 4:00 pm at six equidistant fixed points within each wetland, located 10 cm radially from wetland edges and 10 cm vertically from the wetland substrate surface.

2.4.11 Water quality - influent and effluent sampling and analyses

Triplicate samples were taken from each influent and effluent point during batch flow events. A batch-flow was a water exchange event over system boundaries, when the following sequence of events occurred: 1) water was discharged from polishing wetlands to the wastewater catchment basin, 2) water was discharged from the recirculating wetlands (R1 and R2) to the polishing wetlands (T1-T6), and 3) freshwater was added to the recirculating wetlands. Batch-flow timing and volumes were dependent primarily upon water quality deterioration, evapotranspiration, and other general management conditions. Due to evapotranspiration, 'topping up' the system with reservoir water occurred in many cases independently of batch-flow events in R1-R2 during fish culture trials and in all wetlands in the periods between

polyculture trials. Topping up activities were included in the experimental data in the same fashion as batch flow events (e.g. volumes and nutrient mass balance).

Triplicate samples were collected from the influent and effluent streams using 200 ml, acid washed polyethylene vessels. Two of the three samples were analyzed for total nitrogen, oxidized nitrogen, ammonia, total phosphorus, and FRP (third samples were back-up for nutrient analyses). Samples for oxidized nitrogen, ammonia, and FRP were filtered through Millex ® Millipore 0.45 µm acetate filters at the time of collection. All samples were placed on ice immediately after collection, and then transferred to a freezer until analyses. Samples that required transport were firstly pH adjusted (pH 4.0) using 2-3 drops of 30 % hydrochloric acid to halt biological activity, then packed directly on ice in a 20 L insulated plastic ice chest, and transported overnight to the laboratory for analysis within 24 hours of collection. Samples taken for suspended solids analyses were collected in 2 L polyethylene vessels washed with reverse osmosis water, and analyzed within 2 hours of collection.

Nutrient results were compared to the ANZECC / ARMCANZ (see section 2.1.2) guideline trigger levels for lowland river ecosystems. The term 'no data' was listed under the wetland ecosystem category for the South-east Queensland region, where the wetlands in this studied were located.

2.4.12 Climatic Data

Local climatic conditions (ambient temperature, rainfall, solar radiation, and wind) were collected every 15 minutes using a Campbell Scientific CR10X weather

station mounted on top of the aquaculture shade-house, approximately 4 meters above the ground.

2.4.13 Animal culture water management methods

Continuous recirculation rates of 64.8 m^3 day^{-1} (45 L min^{-1}) were maintained, producing theoretical HRTs of 22 min within R1-R2 fish tanks, and 1.96 hr within corresponding culture wetlands (R1 and R2 wetlands) in trials 1 and 2.

In trial 3, the recirculation rate was increased to 129.6 m^3 day^{-1} (90 L min^{-1}) producing theoretical HRTs of 11.11 min^{-1} within R1-R2 fish tanks, and 0.98 hr^{-1} within corresponding culture wetlands. In trial 3 it was necessary to run a second poly pipe airline into each fish tank to increase oxygenation to support increased fish biomass. In the last month of trial 3 fish culture, system aeration was switched over to an independent 250 watt centrifugal air pump, and airlines were fitted with 6 pumice air diffusers (4 cm length x 1 cm radius) per tank. Air diffusers were not used in trials 1-2.

In all trials, component operating water depths were maintained at 1 m in the fish culture tanks (1 m^3 volume each) and near 40 cm in the recirculating - culture wetlands (4.3 m^3 volume each), and 25 cm in polishing wetlands (899 L volume each). During interim periods between polyculture activities culture wetlands were maintained at water level of *c.* 10 cm depth, and were not populated with fish, although residual juvenile crayfish progeny persisted. In the interim between trials 2 and 3, R1-R2 wetlands were dry fallowed (i.e. wetlands were drained of water and dried out) for two weeks (to facilitate core sampling), and thereafter were free of fish

and / or crayfish inhabitants until trial 3 stocking activities. Water levels were maintained between 5-10 cm in the polishing wetlands over interim periods. Water heating was utilized in R1 - R2 systems only during times of fish culture when water temperatures fell below 30 °C (e.g. cooler seasons, over-night drops in temperature, rainstorms, etc); and thus the heating elements were not used to provide consistent or optimal temperatures for animal growth at all times.

2.4.14 Harvest of *Lates calcarifer* and *Cherax quadricarinatus*

Fish were fasted for 24 h prior to all harvest activities. At harvest, fish were anaesthetized and measured as outlined in section 2.3.7. Three to five fish were dosed with *Aquis* anesthetic (40 ppm), and then examined internally and externally for parasites by Dr. Bret Heath (CQU fish parasitologist). In trial 1, four randomly harvested fish were not anaesthetized, but transferred to ice slurry for 20 minutes, measured, then frozen in block form and sent to FSAS for CNP and dry-weight analyses while in trial 2 five fish were collected and processed in the same fashion.

Crayfish harvest sub-samples were kept in 50 L plastic buckets containing aerated tap water for 24 hrs prior to freeze drying for 3 days at -100 ° C, desiccated for a further 3 days, re-weighed to determine % dry mass, and then sent directly to FSAS for CNP analyses. In trial 1 five of seven sub-samples were analyzed for CNP and in trial 2 ten of ten were analyzed for CNP.

2.4.15 Wetland CNP sampling and analyses

Directly following the trial 2 crayfish harvest, the wetlands were dried via natural evapotranspiration (dry fallowed) to the point where substrates were moist and 'tacky' at the bottom of the wetlands, but before the plants exhibited marked signs of water stress. However, algae at this stage were clearly water stressed, resembling a dry rock-wool or fiber-glass consistency.

Removal of aerial plant matter and algae from wetlands was completed over the period January 5th - 8th, 2003. In wetlands T1-T6 all aerial plant biomass and algal material within the wetlands was collected manually after the drying-out period. In R1 - R2 wetlands, aerial plant and algal biomass was collected from within the core radius (15 cm) only.

Random cores were taken from T1-T6 on January 8th 2003 to quantify soil and plant root biomass, and CNP content after polishing the wastewater of two aquaculture trials. Each sample was taken 20 cm from the wetland edge at randomly chosen "$^{1}/_{8}$ pie sections". Sample core locations in each wetland were as follows: T1 (4,6), T2 (1,2), T3 (7,4), T4 (1,4), T5 (3,6), T6 (6,8). R1 and R2 core sample locations were not randomized as aquaculture solids were expected to settle out in a less uniform fashion given that the fish waste was loaded continually into the culture wetlands from a fixed point source. In R1-R2 wetlands, cores were taken 40 cm in (towards the wetland centre) from the influent and effluent points on the wetland edges.

A steel cylindrical soil corer, 15 cm radius and 75 cm height, was pounded to the bottom of wetland substrates using a sledge-hammer and wooden block top plate.

Each core consisted of all materials within a 15 cm radius of the sample points to the wetland depth of 20 cm. Composite materials were separated manually into soil and root fractions. Methods for the processing of plant and soil sub-samples (weighing, drying, preparation and analysis) for determination of CNP content were as described in sections 2.3.1 and 2.3.4. Collected algae were dried and weighed in the same fashion as was the plant matter with two sub-samples randomly collected from each wetland, secured in plastic vials, and sent with the plant material for CNP analysis.

2.5 Statistical analyses

Within trials 1 - 3, comparisons between wetland experimental treatments refers to the aforementioned polishing wetlands T1-T3 (*Schoenoplectus validus* treatment) and T4 -T6 (*Baumea articulata* treatment).

Culture wetlands R1 and R2 were duplicates of the primary culture system (and not experimental treatments) and provided data on confidence limits for reported mean attributes of the integrated production systems.

A repeated measures analysis of variance (ANOVA) was used to determine whether differences between wetland treatments occurred in the following variables, and over time: wetland plant biomass, CNP mass (soil, plant, and water), wetland *in-situ* electrochemistry, canopy surface area and light penetration, stem photosynthetic rate, and frog density. Independent sample t-tests were used to estimate whether differences in fish and crayfish individual weights were evident between R1 and R2

at stocking and harvest dates; in trial 3, because R1 and R2 stocking and harvests were staggered (see section 2.3.7), those values were compared. Independent sample t-tests were used to determine if differences in wetland macro-invertebrate density and family number occurred between treatments. In addition, a cluster analysis with the Bray-Curtis similarity measure (Clarke and Warwick, 1994a, Clarke and Warwick, 1994b) was used to determine the similarity of benthic macro-invertebrate community composition between wetland treatments and over time. Carbon - nitrogen - phosphorus mass balance calculations were used to determine efficiency in terms of animal production, water usage, and CNP partitioning and travel over system boundaries. In this dissertation, all error bars represent the standard deviation of the set of numbers plotted.

2.6 Results

This section reports the results of experimental trials integrating *Lates calcarifer* culture, *Cherax quadricarinatus* culture, and constructed wetlands.

2.6.1 *Lates calcarifer*

Polyculture of barramundi / red claw integrated with wetlands was repeated over three trials, without statistical differences occurring between R1 and R2 in individual fish from tip of head to bottom of the telson weight at any of the stocking and harvest dates.

At the end of the trials fish feeding rates remained above 2.0 % bw d^{-1}, ranging 2.2 - 2.6 % bw d^{-1} between trials. Fish SGR also remained above 2.0,

ranging 2.3 - 2.8 (Table 2.3). Mean food conversion ratios varied 0.81 - 1.03 : 1.00, remaining below 1.0 over trials 1 and 2. Fish tank culture density ranged 8.1 - 25.2 kg m^3 (1.4 - 2.3 kg m^2 wetland density), producing specimens ranging on average 274.1 g - 520.6 g size (Table 2.3; Figure 2.1). Culture temperatures ranged 27.1 to 27.4 °C. Fish survival remained above 90 % over trials 1 and 2, dropping to 83.9 % in trial 3 (Table 2.3). Wetland R1 required re-stocking due to 100 % mortality caused by pump failure in trial three, and thus the survival percentage reported for

	trial 1 - 2001			trial 2 - 2002			trial 3 - 2003		
	R1	R2	mean	R1	R2	mean	R1	R2	mean
feeding rate - to satiation: (% body weight day^{-1})	2.59	2.63	2.61	2.15	2.29	2.22	2.50	2.18	2.34
SGR	2.77	2.72	2.79	2.56	2.63	2.60	2.52	2.31	2.42
Growth rate (g d^{-1})	3.04	3.00	3.02	3.54	3.38	3.46	1.83	1.84	1.83
FCR	0.80	0.82	0.81	0.81	0.85	0.83	1.14	0.92	1.03
tank culture density (kg m^{-3})	14.8	14.5	14.7	8.5	7.6	8.1	20.2	30.2	25.2
wetland culture density (kg m^{-2})*	1.4	1.4	1.4	0.8	0.7	0.8	1.9	2.8	2.3
individual weight at harvest (g)	386.4	382.9	384.6	533.0	508.3	520.6	266.0	282.2	274.1
culture tank temp °C	27.3	27.5	27.4	27.1	27.1	27.1	27.2	27.3	27.3
survival %	97.5	100	98.8	95	90	92.5	75.4	92.3	83.9
days of fish culture	123	123	123	147	147	147	141	147	144

Table 2.3. *Lates calcarifer* culture efficiency values for three trials: feeding rate, SGR, growth rate, food conversion ratio (FCR), tank culture density, wetland culture density, individual weight at harvest, culture take temperature, survival, and the days of fish culture. Note that the values for some of the variables in Table 2.3 are mean values. Statistical differences were not measured between duplicate culture systems (see section 2.5 – paragraph 2).

* calculation includes the surface are of corresponding culture wetland only; if using all wetlands surface area divide the wetland culture density value listed in the table by 2.

Fish in trial 1 consumed more food and converted it more efficiently, growing faster than fish in trials 2 and 3. The differences between trials 1 and 2 SGRs and FCRs were negligible (SGR difference = 0.19, FCR difference = 0.02), with greater differences occurring between them and trial 3 (SGR difference = 0.29, FCR difference = 0.21). Trial 2 maintained the lowest culture density conditions and individual fish grew larger than in trials 1 and 3. Trial 3 produced the smallest fish under the highest tank densities (Figure 2.1), and achieved the poorest survival rate.

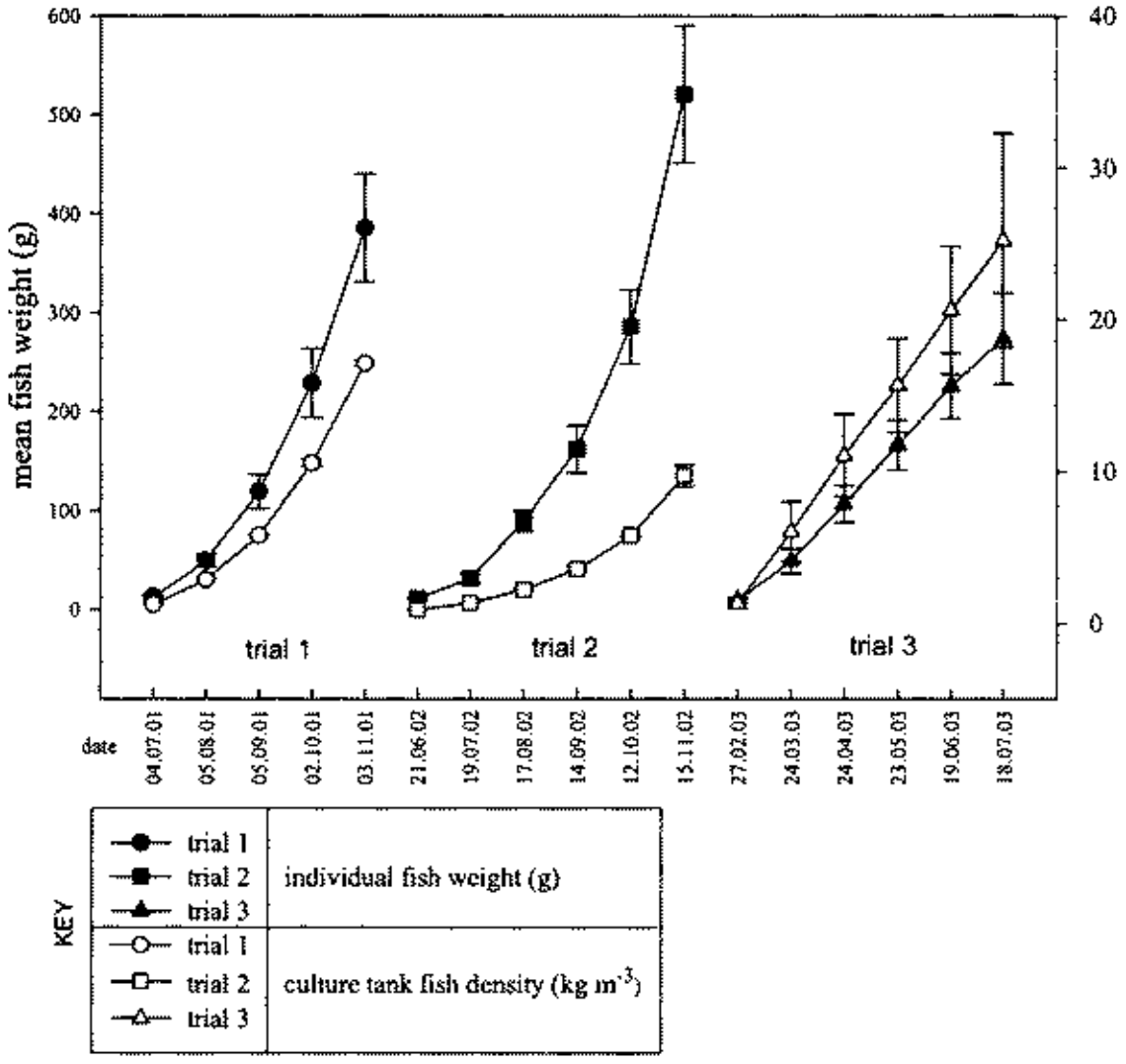


Figure 2.1: Barramundi individual fish weights and culture tank densities over three trials.

A parasite analysis revealed for one of the fish inspected in trial 1 one mite in

the gill cavity attached to a gill filament. The mite was adjacent to an eroded lesion and associated epithelial hypertrophy of filament and lamellae. This lesion was located near other lesions that were all similar in appearance and restricted to a small area that affected the filaments and lamellae of four gills on that side of the fish. Parasites were not found in any other tissues. In trial 2, fish had from 1 to 3 granulomatous cysts ranging in color from white - yellow - brown and in size from 0.25 - 1.0 mm radius within liver mesenteries; fishes 1 - 4 carried 3, 6, 2, and 1 cyst(s) respectively. The cysts were suspected to be of the cestode family, although unable to be positively identified. Parasitic infection was mild in both trials 1 or 2, and was not suspected to have impacted on fish growth.

In trial 3, all five fish examined exhibited early stage protist infection of the gill tissues, due to *Epistylididae rhabdostyla*. If trial 3 harvest was planned for a later date, the fish surely would have shown obvious behavioral signs of infection (such as rejecting or spitting out food) and a salt bath would have been necessary. Each fish had 1 to 7 granulamalous cysts in the liver and in the mesenteries of fish 1-3, the gills in fish 4-5, and in the stomach mesenteries and externally behind the left pectoral fin of fish number 2. Despite being subjected to the early stages of the gill infection as well as a light cestode infection, the fish ate well and did not shows signs of stress; hence the infection was not suspected to have impacted fish growth.

2.6.2 *Cherax quadricarinatus*

Successful *in situ* wetland polyculture of red claw / barramundi occurred in all trials, without statistical differences between R1-R2 mean crayfish weight at

stocking and harvest dates, but with the exception of trial 2 when harvest R1 individual crayfish were heavier than those of R2 (Table 2.4).

wetland	mean harvest weight (g) ± stdv	Statistical result
R1	8.9 ± 2.9	t (175) = 6.21, p < .001
R2	16.4 ± 7.1	

Table 2.4. Trial 2 red claw harvest mean harvest weight and statistical result.

Over the course of the experiment mean crayfish SGRs were similar over trials, ranging 0.92 - 0.97 (Table 2.5). Culture wetland density ranged from 0.04 to 0.20 (at the end of experiment) kg m^2, producing specimens ranging on average 12.5 g - 79.7 g size; while total harvest size ranged 0.5 - 1.8 kg (Table 2.5; Figure 2.2).

Average culture wetland temperatures were 27 °C, 25 °C, and 23 °C in trials 1, 2 , and 3, respectively (Table 2.5). Crayfish in trial 1 maintained the lowest culture density in warmer water, and produced the progeny that were monitored in trial 2. Crayfish in trial 2 grew at higher wetland densities than in trial 1, producing the largest crop by weight but composed of smaller crayfish individuals. Additionally, marked variability between R1 and R2 crayfish SGR and mean weight in trial 2 was evident. Trial 3 produced the largest crayfish which grew faster in cooler temperatures than crayfish in trials 1 and 2, but achieved the lowest survival rates (Table 2.5, Figure 2.2).

	trial 1 - 2001			trial 2 - 2002			trial 3 - 2003		
	R1	R2	mean	R1	R2	mean	R1	R2	mean
specific growth rate (SGR)	0.91	0.92	0.92	0.60	1.24	0.92	0.96	0.98	0.97
growth rate (g d^{-1}) *	0.24	0.29	0.26	0.06	0.04	0.05	0.39	0.41	0.40
wetland crayfish density (kg m^{-2}) ***	.04	0.05	0.04	0.18	0.15	0.16	0.12	0.14	0.10
individual weight at harvest (g)	58.6	70.9	64.8	16.1	8.9	12.5	77.8	81.5	79.7
harvest crop size (kg fresh weight)	0.47	0.50	0.5	1.94	1.56	1.8	1.32	1.55	1.4
mean culture wetland temp °C	27.1	27.3	27.2	25.2	25.2	25.2	23.8	23.3	23.6
survival (%)	80.0	70.0	75.0	**	**	**	63.0	73.1	68.1
days of crayfish culture	211	211	211	146	146	146	153	153	153

Table 2.5. *Cherax quadricarinatus* culture efficiency values for three trials: SGR, growth rate, wetland crayfish density, individual weight at harvest, crop size at harvest, mean culture wetland temperature, survival, and the days of crayfish culture. Note that the values for some of the variables in Table 2.3 are mean values. Statistical differences were not measured between duplicate culture systems (see section 2.5 – paragraph 2).

* calculation made in g d^{-1} units for comparison made to examples in sections 1.12 and 2.7.2.
** number of progeny post trial 1 wetland spawning unknown.
*** calculation includes the surface area of corresponding culture wetland only; if using all wetlands surface area divide the wetland culture density value listed in the Table by 2.

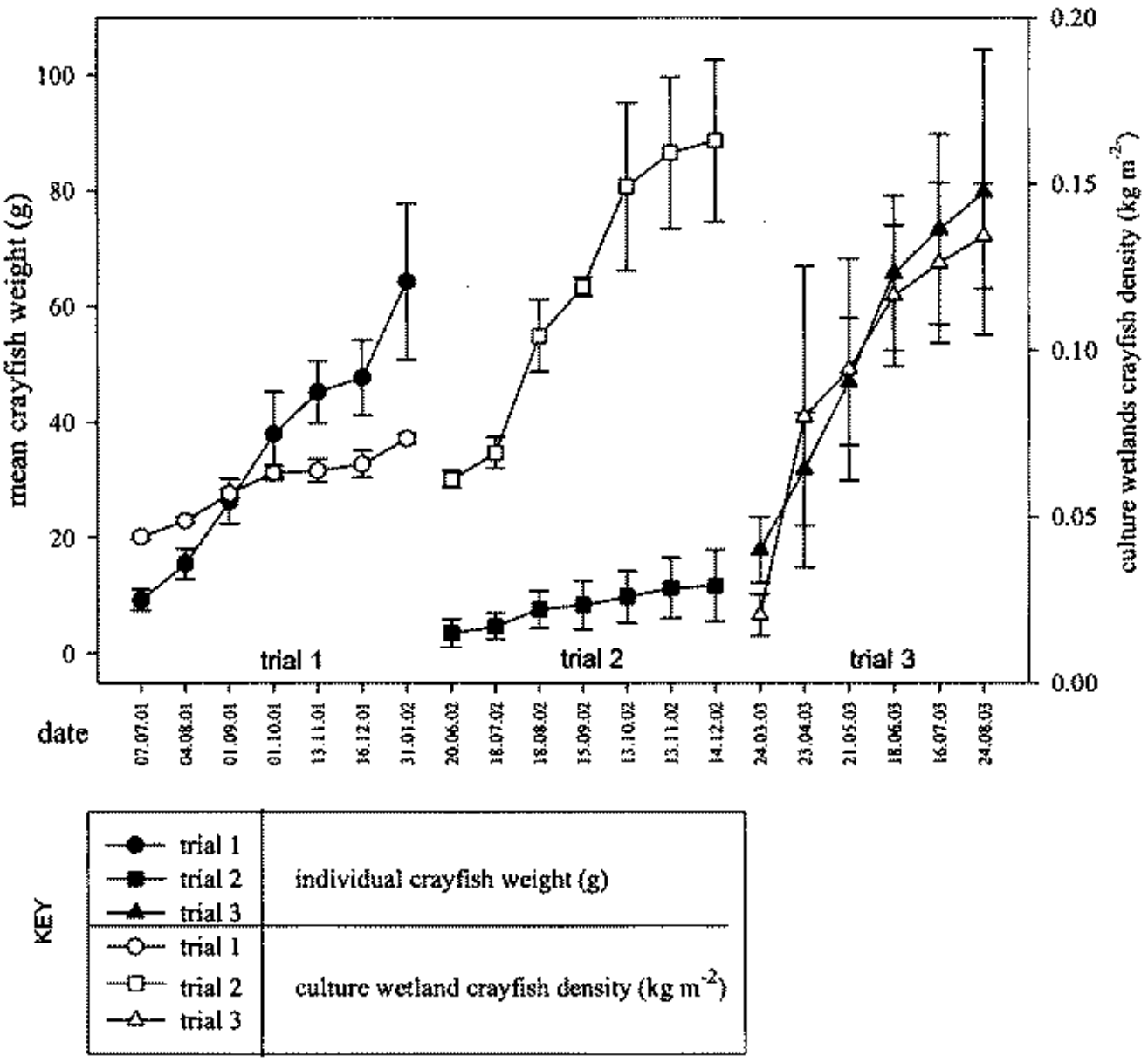


Figure 2.2. Red claw individual fresh weights and wetland culture densities over three trials.

2.6.3 *Velesunio ambiguus*

The mean SGR of mussels over the 175 days period was - 0.06. Populations had suffered 67 % (R1) and 64 % (R1) mortality by the end of trial 1, and had died out by the end of trial 2.

2.6.4 *Litoria fallax*

Baumea articulata polishing wetlands (T4-T6) supported more frogs than *Schoenoplectus validus* polishing wetlands (T1 –T3) ($p < .05$), supporting on average 2 frogs per square meter wetland surface area on the latest sample date (Table 2.6).

	date 19.11.01		date 19.11.02	
wetland	frog number	frog m^{-2}	frog number	frog m^{-2}
T1-T3				
mean	0.33	0.09	2.33	0.65
stdv	0.47	0.13	0.94	0.26
n = 3				
T4-T6				
mean	1.33	0.37	7.33	2.04
stdv	0.58	0.16	1.53	0.42
n = 3				
R1-R2				
mean	40.50	3.77	49.00	4.56
stdv	7.78	0.72	1.41	0.13
n = 2				

Table 2.6. *Litoria fallax* individual juvenile and adult number and wetland density in November 2001 and 2002. *Baumea articulata* polishing wetlands (T4-T6) supported more frogs than *Schoenoplectus validus* polishing wetlands (T1 –T3) ($p < .05$; $n = 3$).

The number of frogs in both wetland treatments increased over time ($p <$

.001) however a significant interaction between time and treatment ($p < .05$), showed that differences between treatments were valid for the second sampling date only (November, 2002). R1 and R2 culture wetland frog populations increased from 3.8 to 4.6 individuals m^{-2} by November 2002. Frogs were observed to feed on dragonfly nymphs as they emerged from the wetlands, and frog proliferation was evident as many individuals at various growth stages (egg, tadpole, and mature) were observed in the wetlands.

2.6.5 Wetland benthic macro-invertebrates

Identity and diversity of macro-invertebrate in the wetland benthic samples are presented in Figure 2.3.

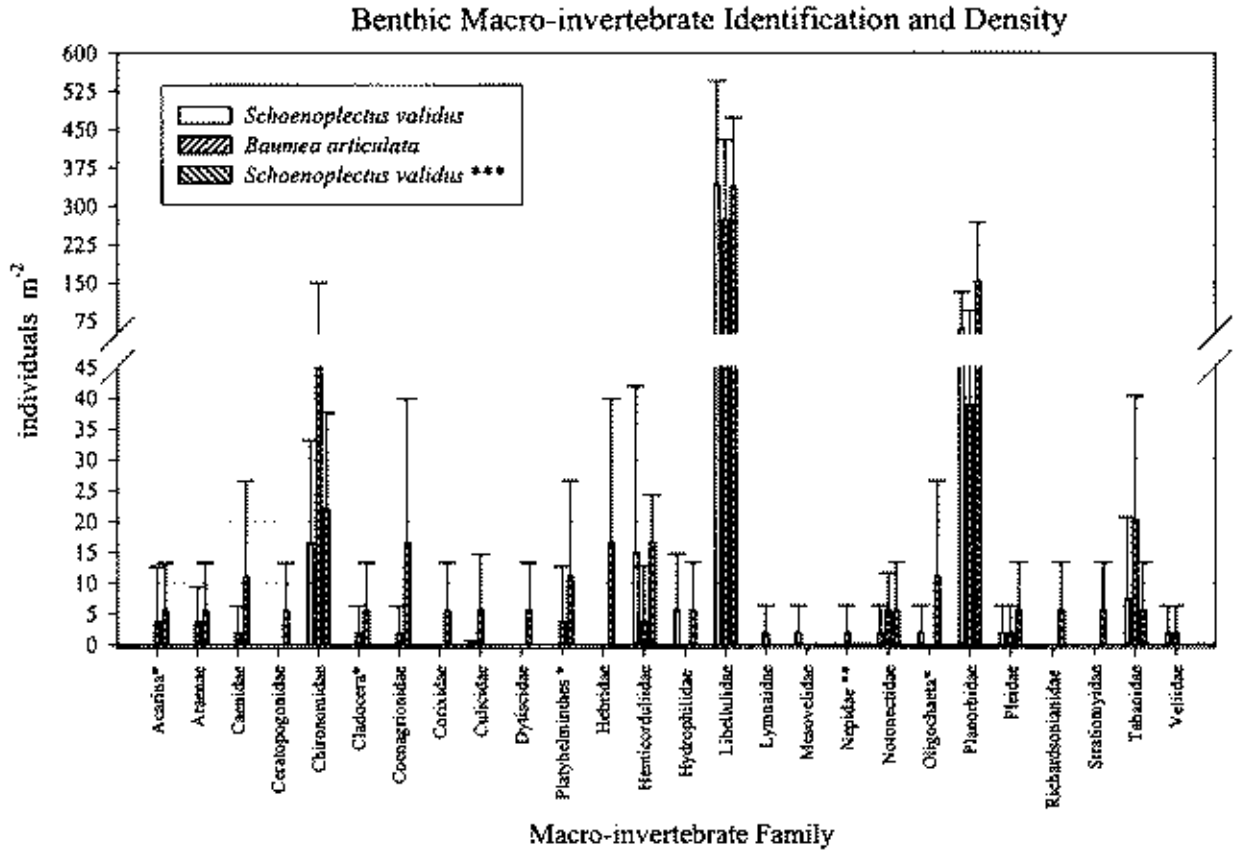


Figure 2.3. Mean density of wetland macro-invertebrate individuals identified by family. * Invertebrate Order, ** Invertebrate Family, *** R1 and R2 wetlands

Eleven orders of macro-invertebrates encompassing 27 families were identified in the wetlands over the experiment. Populations were dominated by Libellulidae (dragonflies and damselflies) and Planorbidae (snails). One sample in T5 wetland was abnormally high in Chironomidae larvae. There were no differences in wetland benthic macro-invertebrate density or family number between treatments. The dendogram generated from the cluster analyses (Figure 2.4) shows that (excepting T5 wetland) the majority of samples taken in 2001 were *c.* 50 % similar to the samples taken in 2002; and that planorbid and libellulid dominance seem to become stronger in the latter year.

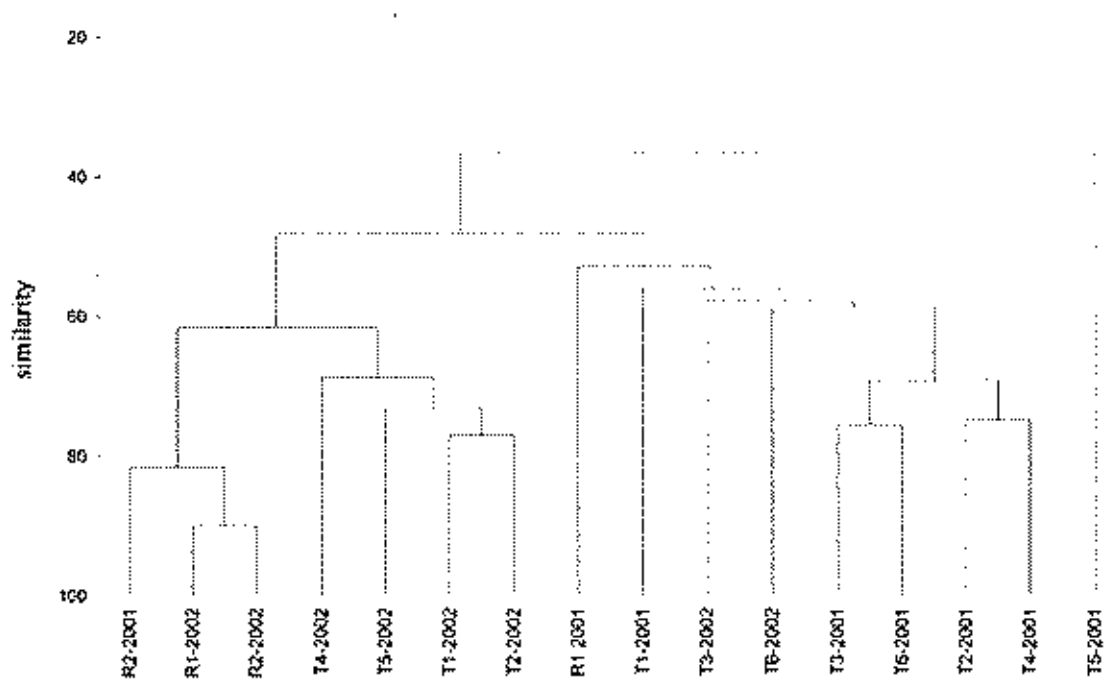


Figure 2.4. Wetland macro invertebrate similarity dendogram

2.6.6 *Schoenoplectus validus* and *Baumea articulata*

Statistical difference in wetland dry weight plant biomass between wetland treatments (T1-T3 and T4-T6) was valid for harvest values only. At the end of the trial 2 the dry weights of *Baumea articulata* (T1-T3) wetland plants were significantly greater ($p < .01$) than those of *Schoenoplectus validus* (T4-T6) wetland plants. Mean RGR for plant biomass of *Baumea articulata* was 0.0043 resulting in an average dry weight harvest of 573 g m^{-2} (Figure 2.5) whereas mean RGR for plant biomass of *Schoenoplectus validus* was 0.0040 resulting in an average dry weight harvest of 211 g m^{-2} (Figure 2.5). Polishing wetland plant biomass increased over time in both *Baumea articulata* and *Schoenoplectus validus* treatments ($p < .001$). Mean RGR for plant biomass of culture wetlands (R1-R2) *Schoenoplectus validus* was 0.0057 RGR leading to an average dry weight harvest of 7,613 g m^{-2} (Figure 2.5).

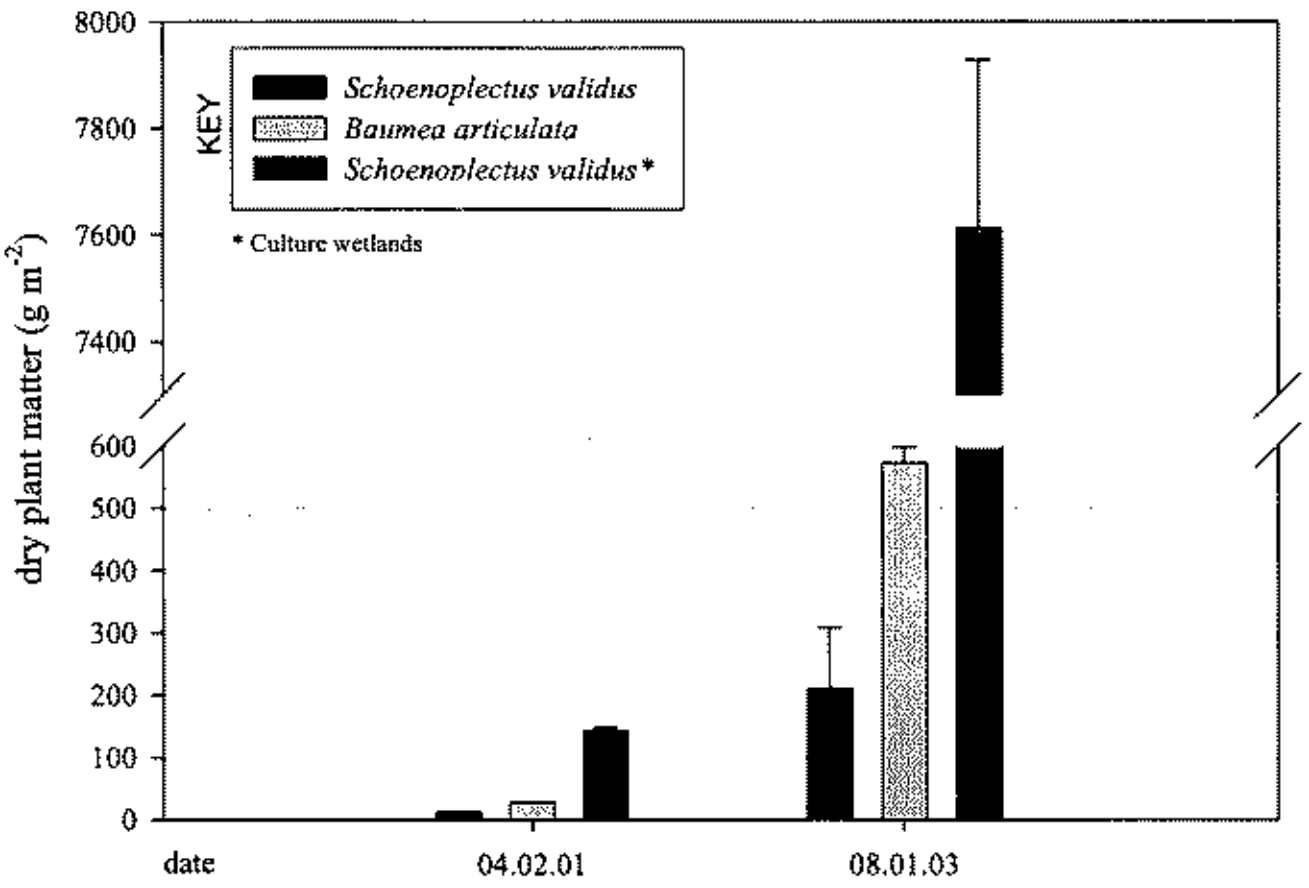


Figure 2.5. Wetland plant biomass at the time of planting and harvest
* Culture wetlands

Statistical difference in wetland canopy surface area between treatments was evident for harvest values only. Plant canopies of *Baumea articulata* wetlands had significantly greater surface area than those of *Schoenoplectus validus* plants at harvest ($p < .01$). Mean surface area of *Baumea articulata* wetland canopies increased by 0.39 relative surface area expansion rate (RSR) over 703 days to an average of 1.539 m^2 of canopy surface area per wetland area (m^2) at the time of harvest (Figure 2.6); while the surface area of *Schoenoplectus validus* wetland canopies increased by 0.42 RSR to an average of 0.494 m^2 per wetland area (m^2) at the time of harvest. Mean surface area of culture wetland *Schoenoplectus validus*

canopies increased by 0.53 RSR to an average of 18.148 m^2 per wetland area (m^2) at the time of harvest (figure 2.6).

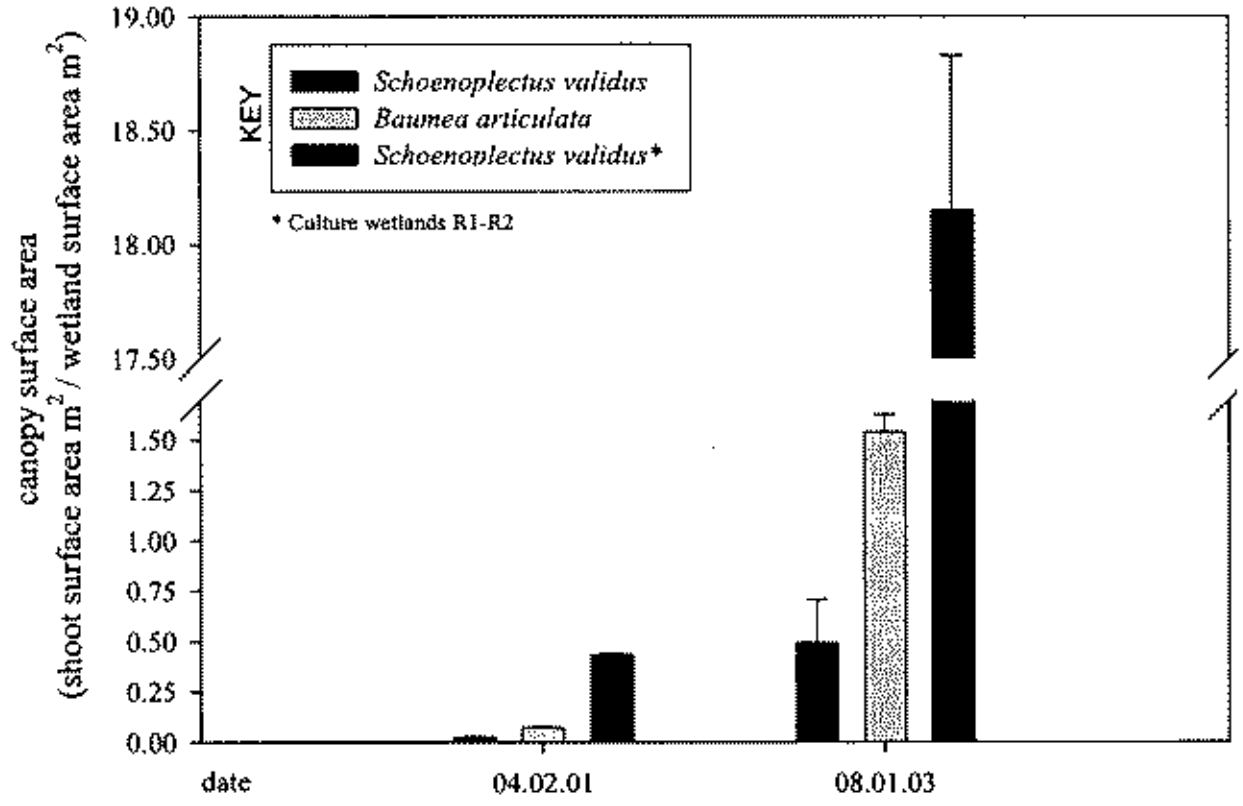


Figure 2.6. Wetland plant canopy surface area at the time of planting (04.02.01) and harvest (08.01.03).

Photosynthetically active radiation measured at water-level (W-PAR) for *Baumea articulata* was significantly lower than for *Schoenoplectus validus* ($p < .01$) on all but the first two sampling dates May and June 2001. Mean *Baumea articulata* relative light penetration decrease rate (RLDR) was 0.09 indicating interception of 35 % of corresponding above-canopy photosynthetically active radiation (A-PAR) at the time of harvest, whereas RLDR for *Schoenoplectus validus* W-PAR was 0.03 leading to 9 % canopy absorption of PAR (Figure 2.7). Average RLDR for culture wetlands (R1 - R2) was 0.24 with 74.1 % absorption of PAR at the time of harvest (Figure 2.7).

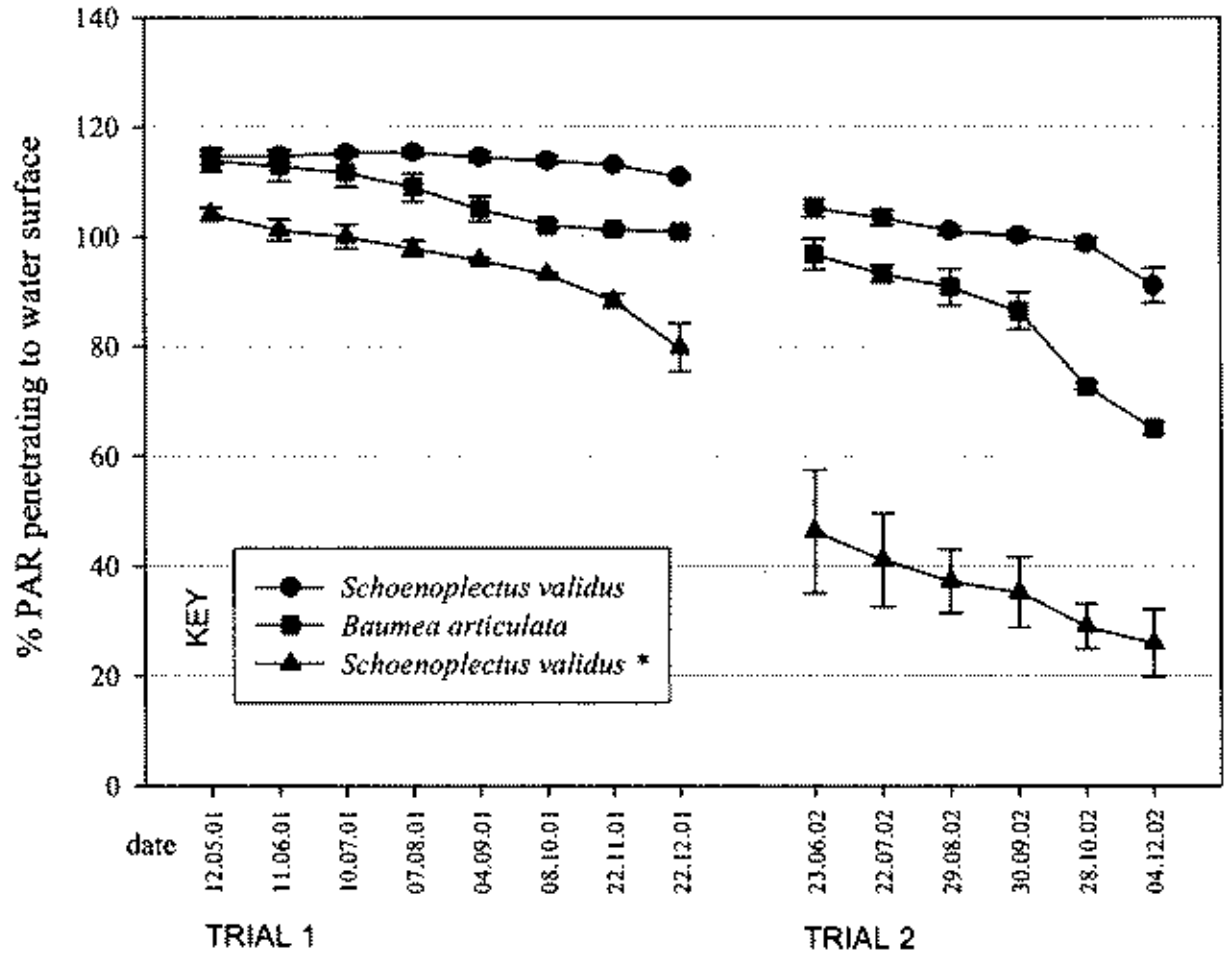


Figure 2.7. Wetland water-level photosynthetically active radiation (W-PAR) (i.e. % of photosynthetically active radiation penetrating to the waters surface). * Culture wetlands R1-R2

The rate of net photosynthesis measured for *Baumea articulata* growing in T4 - T6 polishing wetlands was significantly higher than for *Schoenoplectus validus* plants growing in polishing wetlands T1-T3 on the first and third sampling dates only ($p < .05$). *Baumea articulata* net photosynthetic rates were 7.23 μmol m^{-2} sec^{-1} in trial 1 and 6.33 μmol m^{-2} sec^{-1} in trial 2, while *Schoenoplectus validus* net photosynthetic rates were 6.48 μmol m^{-2} sec^{-1} in trial 1, and 5.98 μmol m^{-2} sec^{-1} in trial 2 (Table 2.7 ; Figure 2.8). At mean canopy surface area achieved (*Baumea articulata* wetlands = 1.54 m^2, *Schoenoplectus validus* wetlands = 0.49 m^2) and mean net photosynthetic rates, *Baumea articulata* and *Schoenoplectus validus* wetlands could, on average, assimilate 5.90 and 1.84 g CO_2 hr^{-1}, respectively, during peak

illumination hours (i.e. 11: 00 am to 1:00 pm).

	trial 1	trial 2				
wetland	plant CO_2 absorption ($\mu mol\ m^{-2}\ sec^{-1}$)	plant CO_2 absorption ($\mu mol\ m^{-2}\ sec^{-1}$)	Trials 1-2	wetland canopy surface area (m^2)	Wetland CO_2 absorption ($mol\ hr^{-1}$)	Wetland CO_2 absorptio ($g\ hr^{-1}$)
S. validus						
mean	6.48	5.98	6.42	1.77	0.041	1.84
stdv	0.10	0.34	0.22	0.78	0.022	0.95
B. articulata						
mean	7.23	6.33	6.75	5.52	0.139	5.90
stdv	0.32	0.30	0.31	0.31	0.009	0.38
*S. validus**						
mean	10.14	7.35	8.75	195.09	6.143	270.31
stdv	0.01	0.12	0.07	7.25	0.188	8.26

Table 2.7. Plant and total wetland photosynthetic rate. Measured photosynthetically active radiation was significantly lower in *Baumea articulata* was wetlands ($p < .01$; $n = 3$) on all but the first two sampling dates May and June 2001.

The average net photosynthetic rate of culture wetland plants was 10.14 in trial 1 and 7.35 $\mu mol\ m^{-2}\ sec^{-1}$ in trial 2. At maximum canopy surface area and mean net photosynthetic rate (Table 2.7), each culture wetland could, on average, assimilate 270.31 g $CO_2\ hr^{-1}$ during peak illumination hours. The last set of culture wetland data in trial 1 was not collected due to equipment failure.

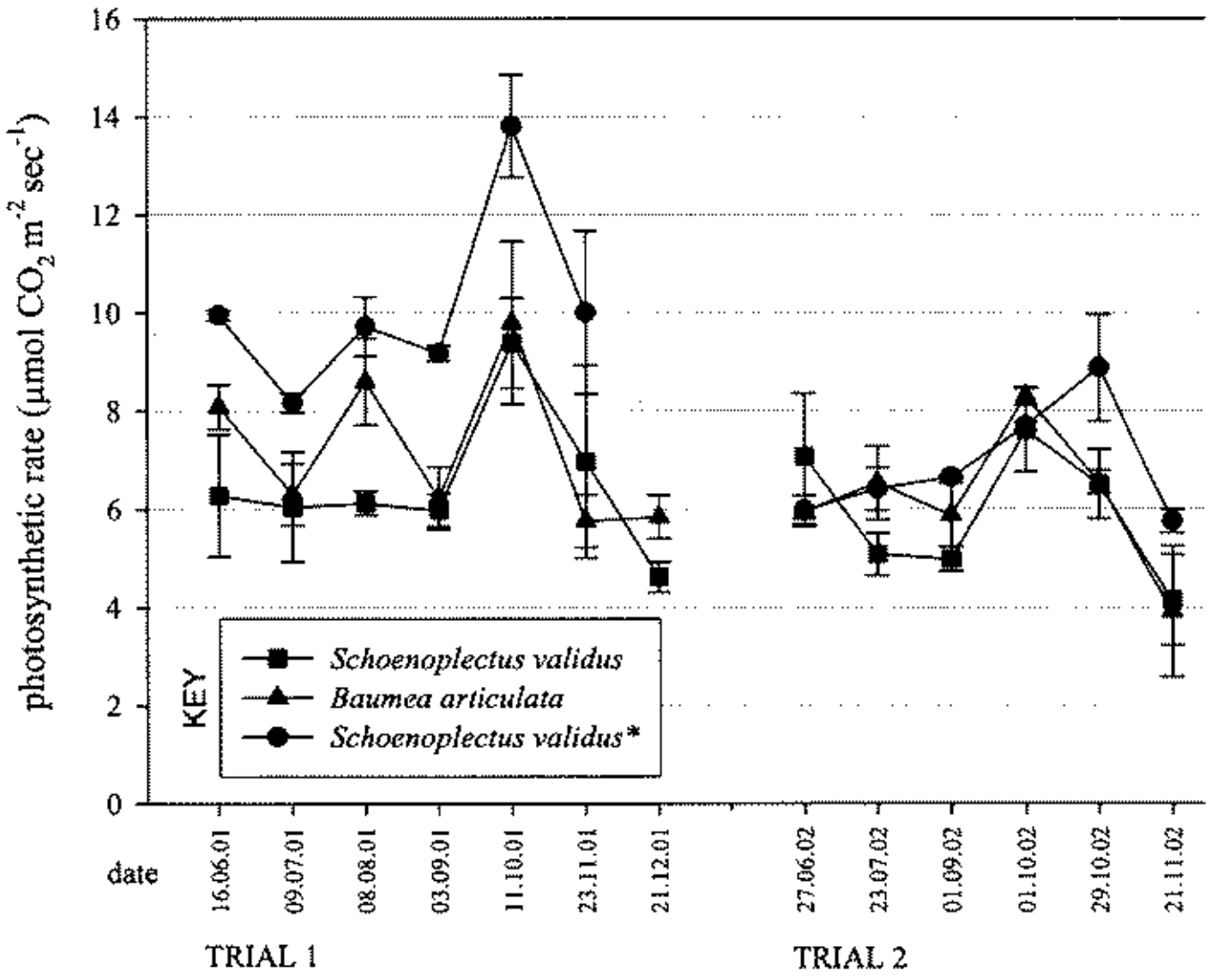


Figure 2.8. Plant photosynthetic rate.

* Culture wetlands R1-R2

Table 2.8 shows the planting and harvest CNP for *Schoenoplectus validus* and *Baumea articulata*. The carbon concentration of polishing wetland plant samples was not significantly different between species, and did not significantly change from planting to harvest.

Author	planting or harvest	Species	system	sample	carbon (mg g^{-1})	nitrogen (mg g^{-1})	phosphorus (mg g^{-1})
Roe (2004)	planting	*S. validus*	wetlands R1-R2	AG	440.16	3.70	0.18
				BG	401.25	7.92	0.40
			wetlands T1-T3	AG	367.73	9.25	3.27
				BG	309.15	11.56	2.96
		B. articulata	wetlands T4-T6	AG	400.42	7.88	0.79
				BG	423.91	8.71	0.57
	harvest	*S. validus*	Wetlands R1-R2	AG	431.14	13.79	1.44
				BG	266.73	8.88	1.73
			wetlands T1-T3	AG	412.08	7.26	0.71
				BG	411.50	7.36	1.10
		B. articulata	wetlands T4-T6	AG	427.20	4.71	0.30
				BG	298.68	4.94	0.60

Table 2.8. Carbon, nitrogen, and phosphorus concentrations of *Schoenoplectus validus* and *Baumea articulata* samples taken at planting and harvest. AG = above ground biomass; BG = below ground biomass. Note: see digital appendix 1 – folder mass balance – files K and L for detailed data on plant sample nitrogen and phosphorus analysis. The carbon and phosphorus concentrations of polishing wetland plant samples was not significantly different between species ($p > .05$; $n = 5$). *Schoenoplectus validus* polishing wetland samples had significantly higher nitrogen concentrations than the *Baumea articulata* polishing wetland samples ($p < .01$; $n = 5$).

Concentrations of nitrogen decreased significantly from planting to harvest time in both *Schoenoplectus validus* and *Baumea articulata* polishing wetland plant samples ($p < .05$). *Schoenoplectus validus* polishing wetland samples had consistently higher nitrogen concentrations than the *Baumea articulata* polishing wetland samples ($p < .01$) on both planting and harvesting sampling occasions. At initial planting, polishing wetland S*choenoplectus validus* samples were more phosphorus rich than those of *Baumea articulata* ($p < .01$). Over time the concentrations of phosphorus in polishing wetland samples decreased significantly in *Schoenoplectus validus* plant samples ($p < .01$), while they increased significantly in *Baumea* articulata polishing wetland plant samples ($p < .01$). At harvest time there

was no difference in the concentration of phosphorus between polishing wetland *Schoenoplectus validus* and *Baumea articulata* plant samples ($p > .05$).

2.6.7 Wetland *in-situ* electrochemical water quality

In trials 1and 2, dissolved oxygen in culture wetlands (R1-R2) ranged from 3.8 - 9.0 mg L^{-1}, and averaged 6.33 mg L^{-1}. Dissolved oxygen concentrations remained below saturation (i.e. amount of oxygen pure water at given salinity, temperature, and barometric pressure) in all but the first data set irrespective of temperature trends and intermittent electrical heating (Figure 2.9).

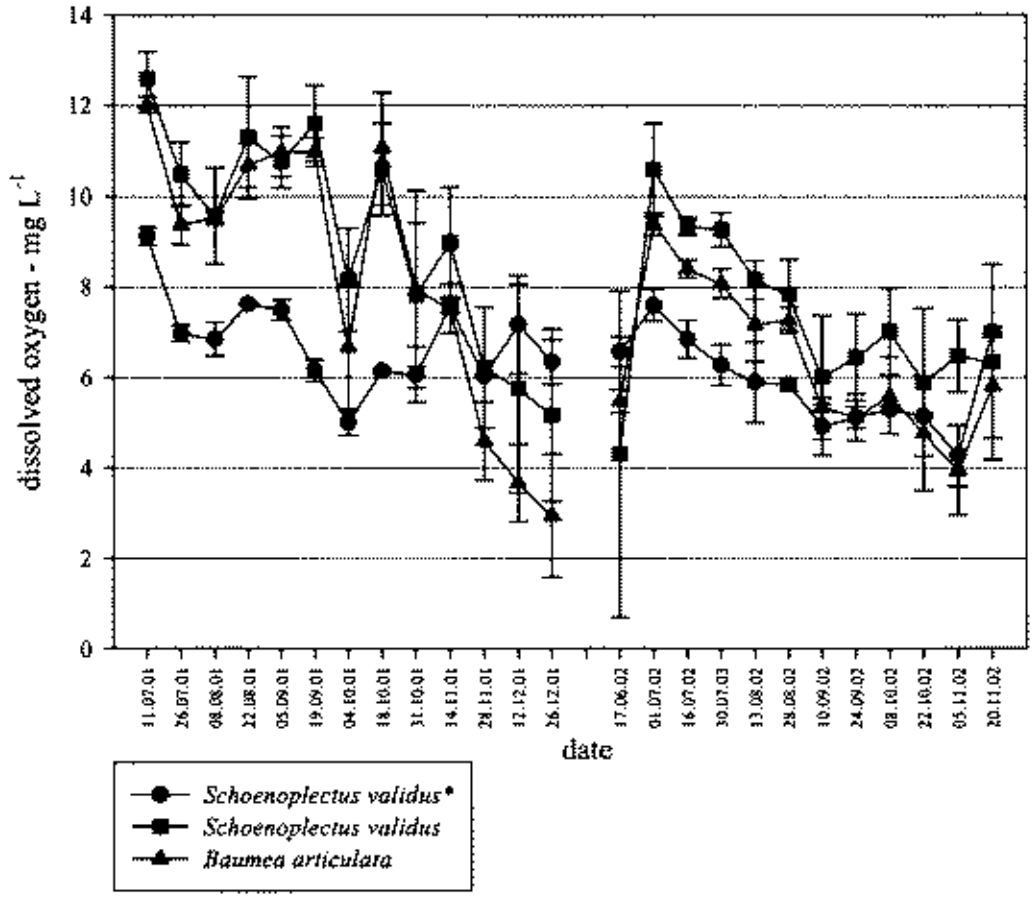


Figure 2.9. Wetlands *in-situ* dissolved oxygen.

* Culture wetlands R1-R2

The pH of culture wetlands ranged 7.2 - 9.5, and averaged 8.1 (Figure 2.10).

The variability of pH between R1 and R2 tanks, and fluctuations from one sample date to the next, were greater in trial 1 than in trial 2 (Figure 2.10). Total dissolved solids in culture wetlands ranged 159.5 - 1684.2 mg L^{-1}, and averaged 720.7 mg L^{-1} (Figure 2.11). Concentrations steadily rose to *c.* 500 mg L^{-1} in the 6th week of the first fish culture in trial 1 (August 2001), and maintained concentrations of 500 - 600 mg L^{-1} until a sharp increase was measured in the 5th week of fish culture in the 2nd trial (August 2002).

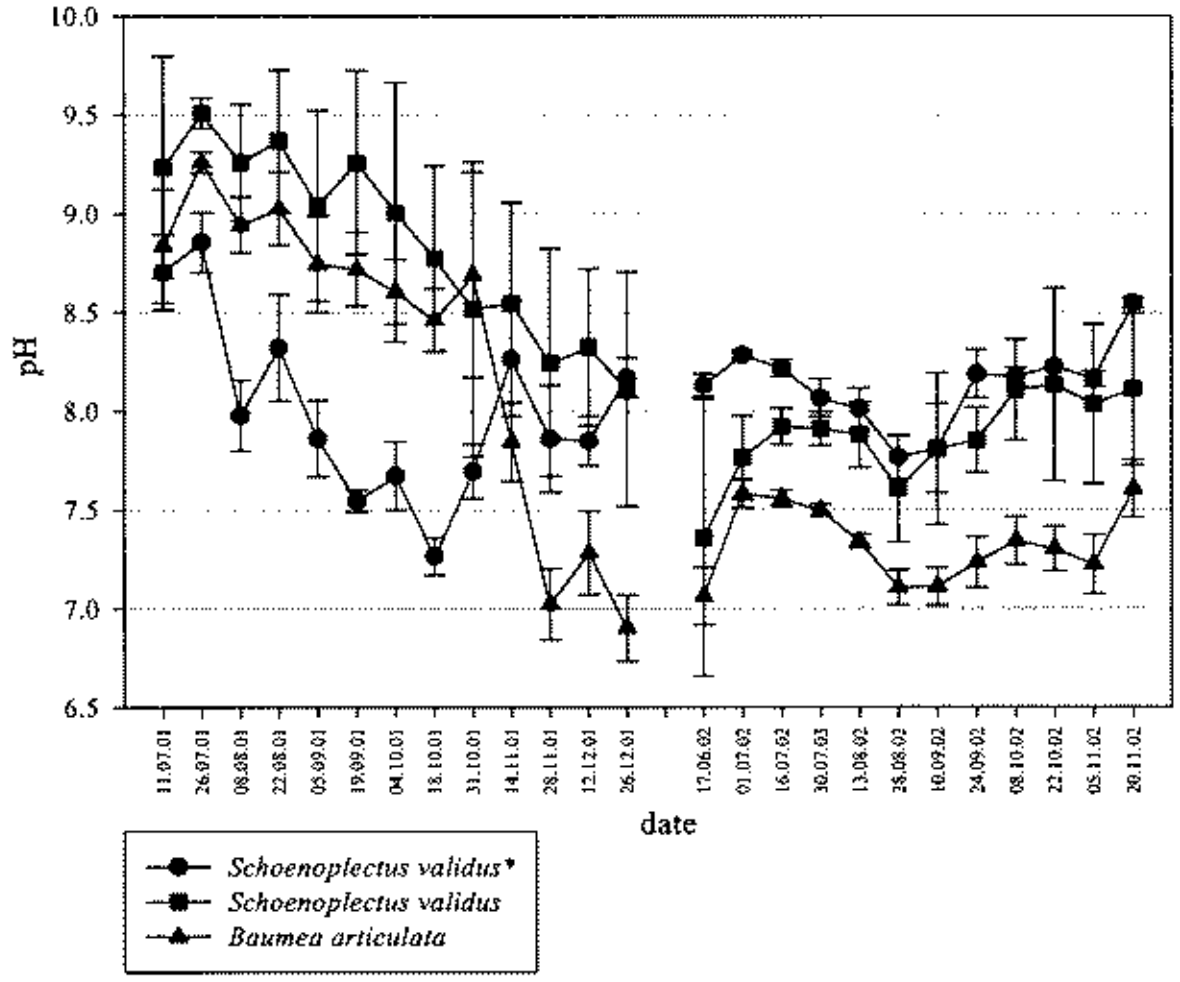


Figure 2.10. Wetlands *in-situ* pH

* Culture wetlands R1-R2

The water temperature of culture wetlands ranged 19.2 - 33.1 °C, averaging 27.1 °C over trials 1 and 2 (Figure 2.12), however water temperatures followed

seasonal patterns buffered to some extent by electrical heating elements that were activated whenever water temperatures were ≤ 30 °C.

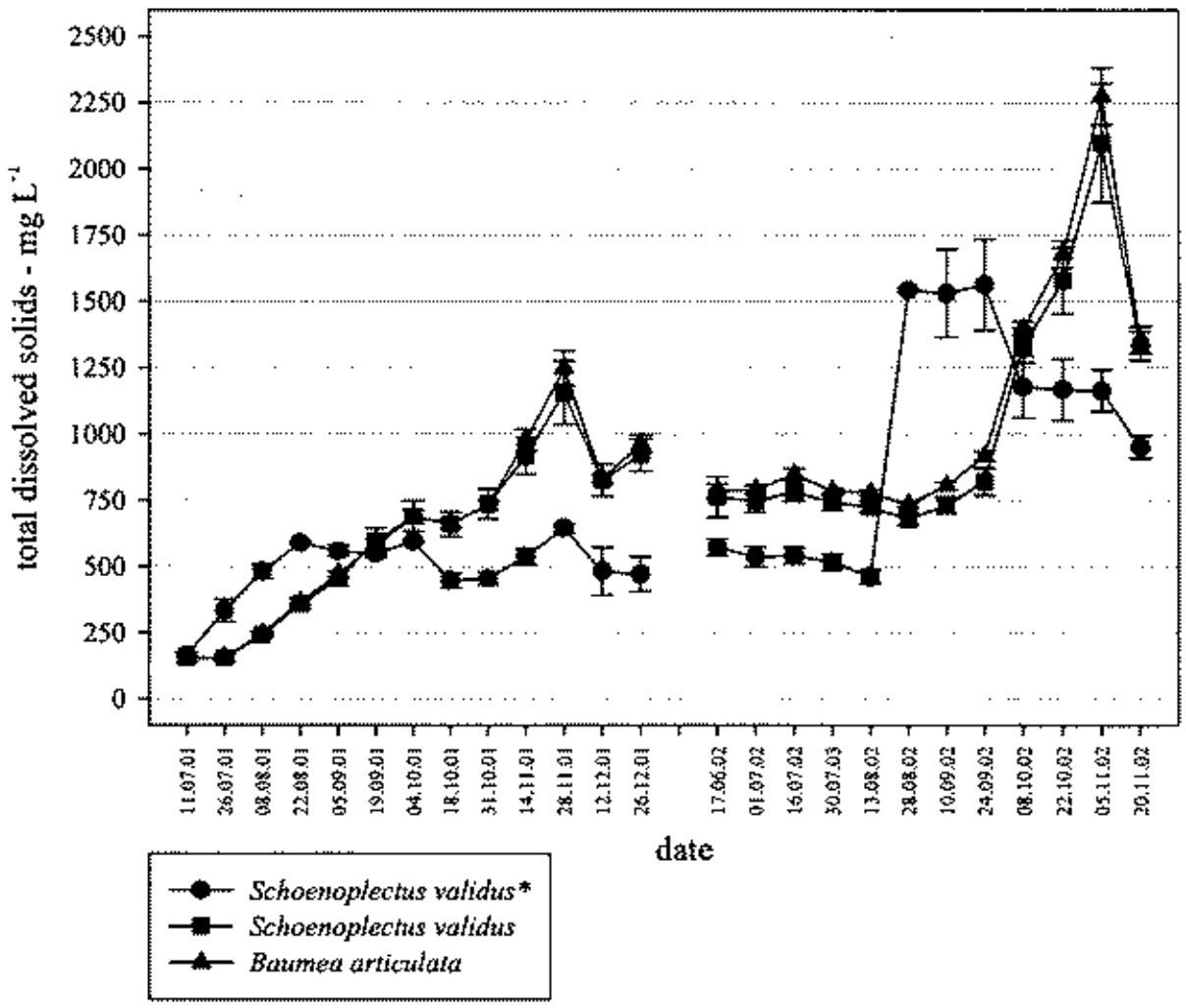


Figure 2.11. Wetlands *in-situ* total dissolved solids.

* Culture wetlands R1-R2

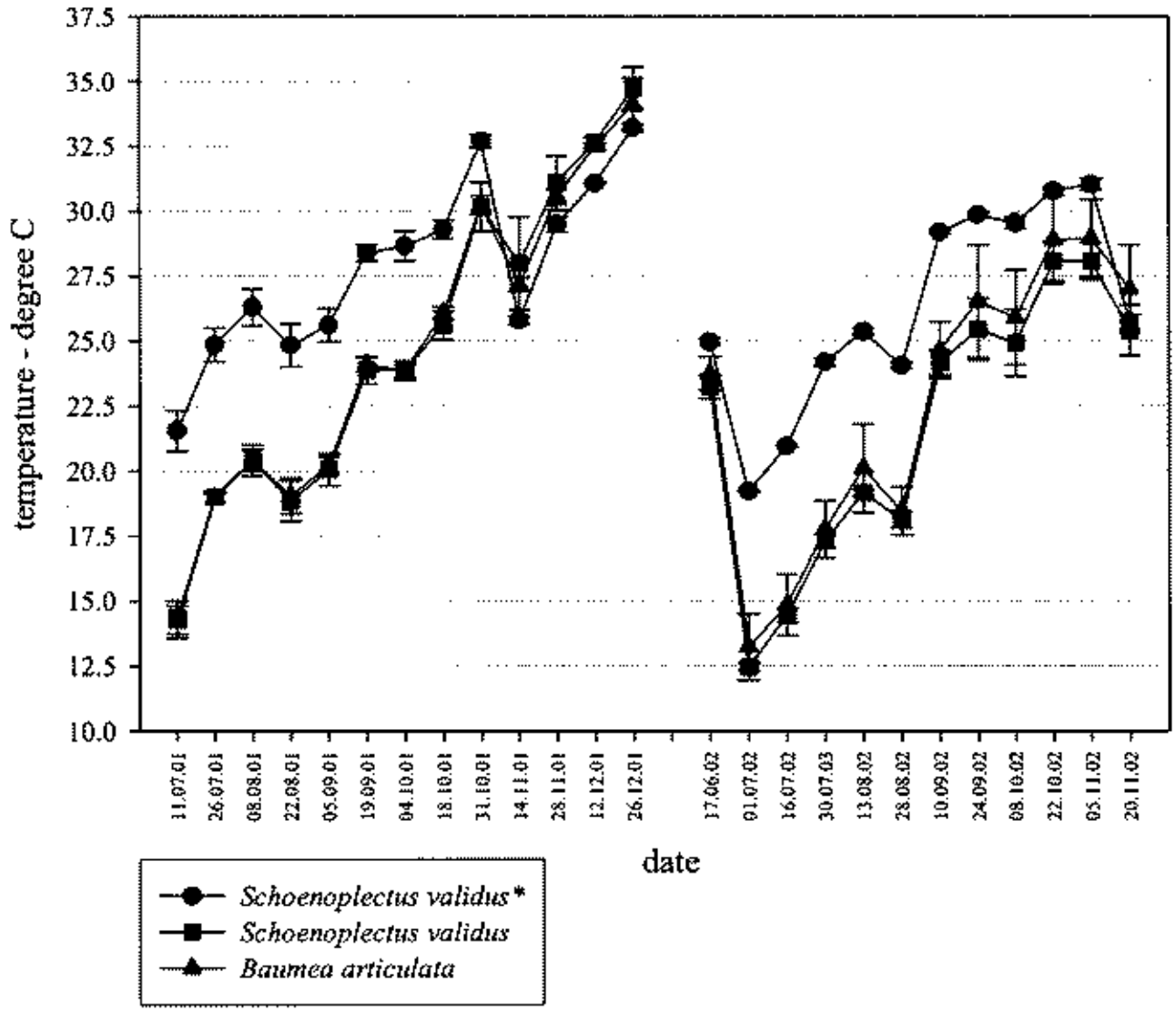


Figure 2.12. Wetlands *in-situ* water temperature (degrees Celcius).

* Culture wetlands R1-R2

Polishing wetlands with *Baumea articulata* plants had significantly lower pH than *Schoenoplectus validus* wetlands ($p < .01$) (Figure 2.10) although dissolved oxygen, total dissolved solids, and temperature refer to figures here did not differ between treatments ($p > .05$). The effect of time was significant across treatments over all electrochemical variables ($p < .01$), and there was no interaction with the main effects ($p > .05$). Over the period of trial 1 mean polishing wetland dissolved oxygen and pH decreased, and total dissolved solids and temperature increased.

Over the period of trial 2, dissolved oxygen decreased, pH fluctuated between 7.0 and 8.25, and total dissolved solids and temperature increased. Polishing wetlands with *Baumea articulata* exhibited dissolved oxygen values in the range of 3.1 - 12.4 mg L^{-1}, averaging 7.4 mg L^{-1}, and which inversely followed water temperature trends (Figures 2.9 and 2.12). The pH of *Baumea articulata* polishing wetlands ranged 6.8 to 9.3, and averaged 7.9. The variability of pH between replicates, and fluctuations from one sample date to the next, were greater in trial 1 than in trial 2 (Figure 2.10). *Baumea articulata* polishing wetlands exhibited total dissolved solids in the range 136.8 - 2407.5 mg L^{-1}, and averaged 849.5 mg L^{-1}. Total dissolved solids concentrations increased from initiation to the conclusion of both trials 1 and 2 (Figure 2.11). *Baumea articulata* polishing wetlands water exhibited water temperatures in the range 12.5 - 32.6 °C, and averaged 23.4 °C while following seasonal patterns (Figure 2.12).

Polishing wetlands with *Schoenoplectus validus* recorded dissolved oxygen values that ranged 2.3 to 13.2 mg L^{-1}, averaged 8.3 mg L^{-1}, and inversely followed water temperature trends (Figures 2.9 and 2.12). The pH of *Schoenoplectus validus* polishing wetlands ranged 6.2 to 9.8, and averaged 8.4. Diminishing variability in pH between replicates, and fluctuations from one sample date to the next over trials 1 and 2, were not as apparent as in the *Baumea articulata* wetlands (Figure 2.10). Total dissolved solids were recorded in the range 139.5 - 2240.8 mg L^{-1}, and averaged 807.5 mg L^{-1} (Figure 2.11). Total dissolved solids concentrations increased from initiation to the conclusion of both trials 1 and 2 (Figure 2.11). *Schoenoplectus validus* polishing wetlands exhibited water temperatures in the range 12.3 - 32.8 °C, and averaged 23.4 °C (Figure 2.12) while following seasonal patterns.

In trial 3, dissolved oxygen concentrations in culture wetlands ranged 4.6 to 8.4 mg L^{-1} (averaged 5.9 mg L^{-1}) and were lower than those of trial 1 and 2, consistently remaining below saturation irrespective of ambient temperature patterns (Figure 2.13).

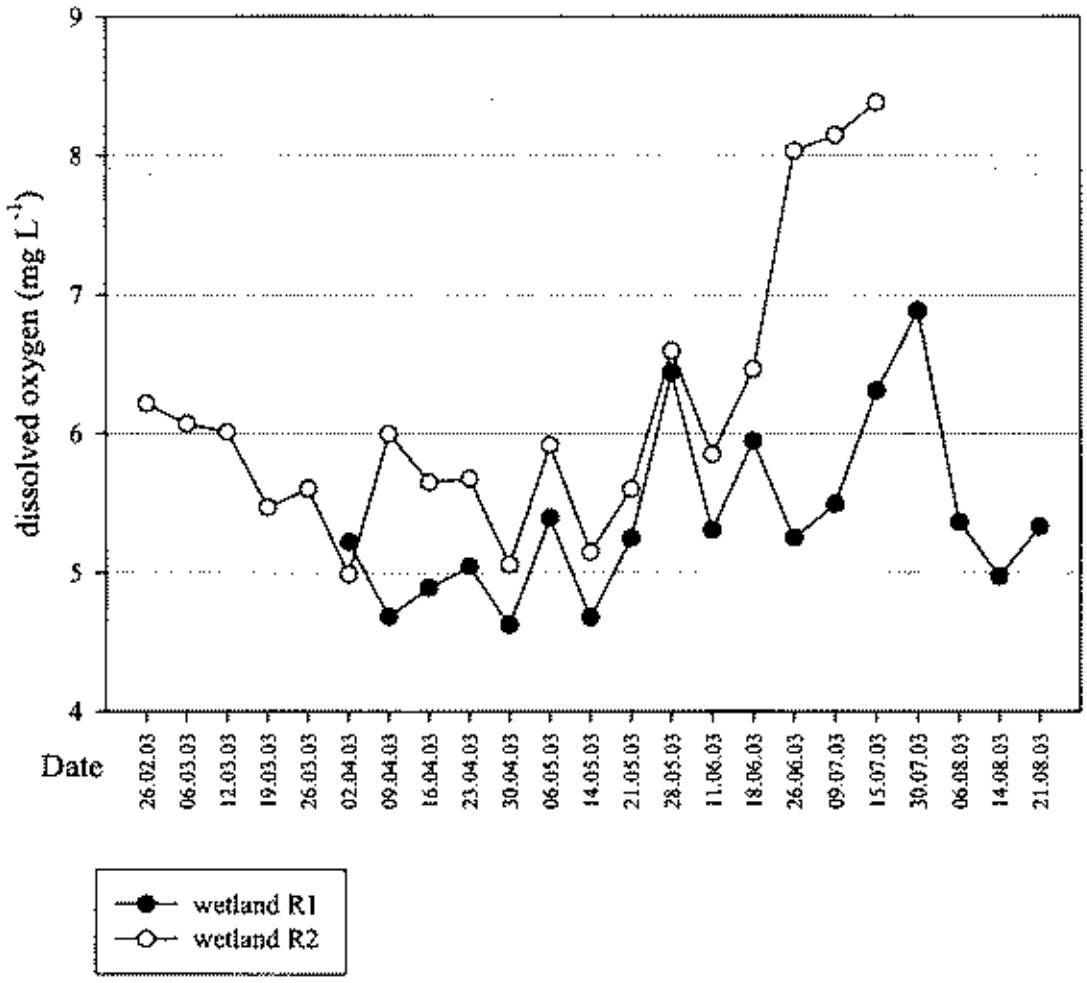


Figure 2.13. Trial 3 - R1 and R2 culture wetlands *in-situ* dissolved oxygen

The pH of culture wetlands ranged 6.8 to 8.3, averaged 7.5, and was lower in trial 2 than trial 1. (Figure 2.14). Total dissolved solids (TDS) ranged 147.2 to 444.4 mg L^{-1}, and averaged 280.1 mg L^{-1}. Wetland R1 culture system TDS increased over trial 3, while R2 TDS peaked in June 2003, then decreased to a harvest TDS concentration of *c.* 200 mg L^{-1} (Figure 2.15).

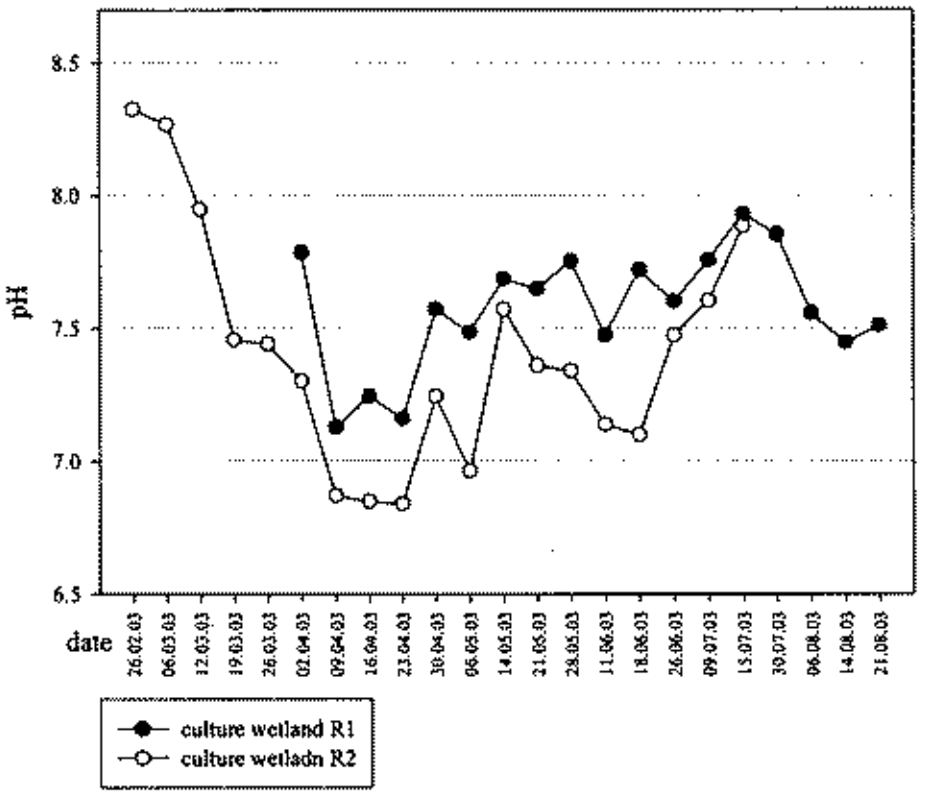


Figure 2.14. Trial 3 - R1 and R2 culture wetlands *in-situ* pH

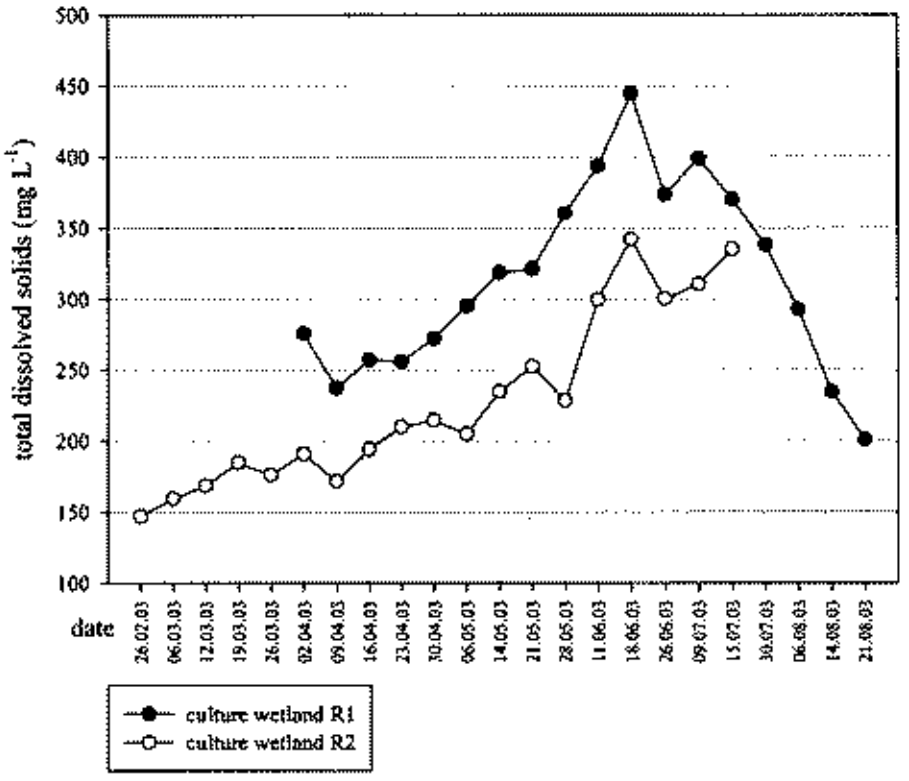


Figure 2.15. Trial 3 - R1 and R2 culture wetlands *in-situ* total dissolved solids.

Culture wetland water temperatures in trial 3 ranged 8.6 to 32.1 °C, averaged 25.4 °C (Figure 2.16), and followed seasonal patterns buffered to some extent by electrical heating elements that were activated whenever water temperatures fell below 30 °C. Dual electrical faults occurred, cutting heating off in June (R2) and July (R1) 2003 (Figure 2.16).

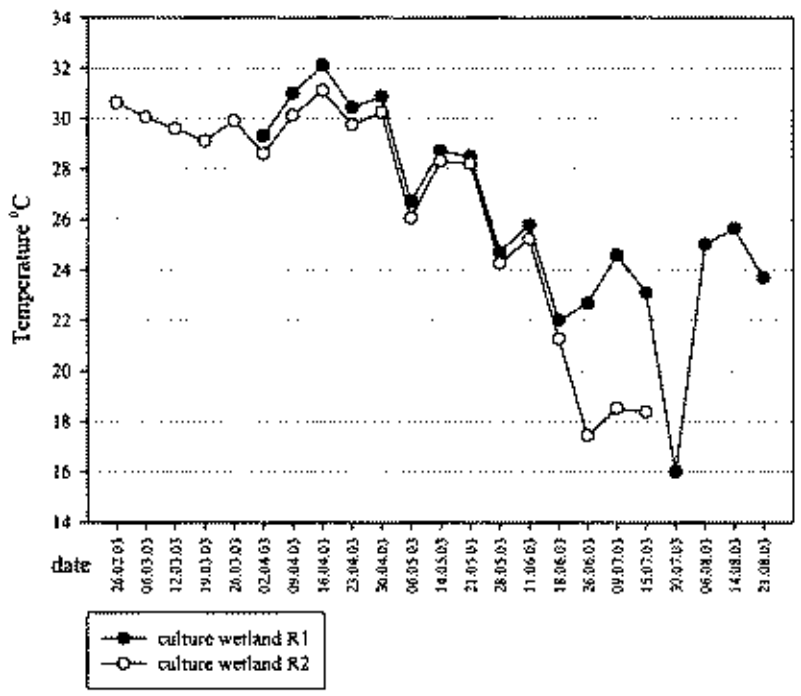


Figure 2.16. Trial 3 - R1 and R2 culture wetlands *in-situ* temperature (degrees Celcius).

2.6.8 Wetland nutrient water quality

Source tap water TN concentration ranged from 7 μg L^{-1} to 1.36 mg L^{-1}, averaging 306 μg L^{-1} over trials 1 and 2, and breeched the ANZECC / ARMCANZ (2000) water quality trigger value (500 μg L^{-1}) 22 % of the time (Figure 2.17). Tap water TP ranged from zero to 148 μg L^{-1}, averaged 38 μg L^{-1}, and breeched the ANZECC / ARMCANZ (2000) trigger value (50 μg L^{-1}) 22 % of the time (Figure 2.20). Tap water suspended solids (SS) concentrations ranged from 0.2 to 0.5 mg L^{-1}, averaged 0.4 μg L^{-1}, and never breached the ANZECC / ARMCANZ (2000) trigger level (6 mg L^{-1}) (Figure 2.22).

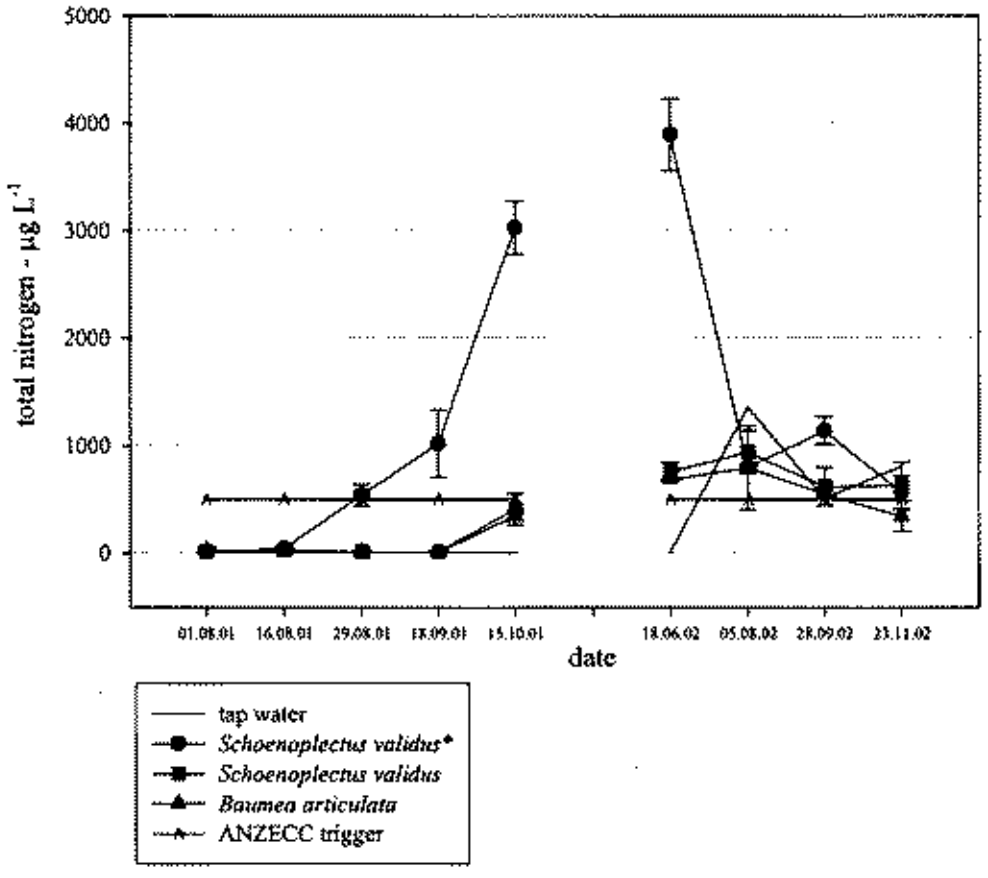


Figure 2.17. Wetland influent and effluent total nitrogen concentration. In this figure, culture wetland influents refer to tap water, and polishing wetlands influents refer to culture wetland effluents. * wetland R1-R2

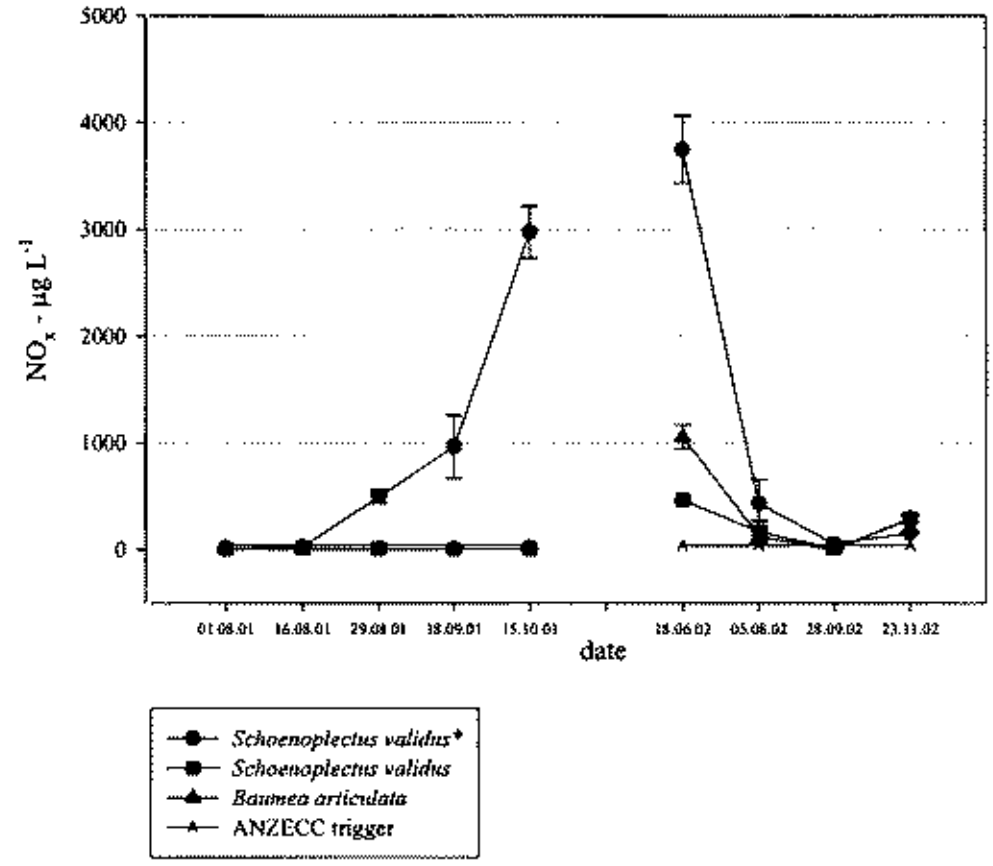


Figure 2.18. Wetland influent and effluent oxidized nitrogen concentration. In this figure, culture wetland influents refer to tap water, and polishing wetlands influents refer to culture wetland effluents. * wetland R1 – R2

The total nitrogen (TN) concentration from effluent from culture wetlands (R1-R2) ranged from 12 μg L^{-1} to 3.89 mg L^{-1}, averaging 1.23 mg L^{-1} over trials 1 and 2 (Figure 2.17). Oxidized nitrogen (NO_x) concentrations ranged 6 μg L^{-1} to 3.75 mg L^{-1}, averaging 984 μg L^{-1}; and ammonium (NH_4^+) ranged from 6 μg L^{-1} to 650 μg L^{-1}, averaging 124 μg L^{-1} (Figures 2.18 - 2.19). Culture wetland effluent concentrations of TN, NO_x, and NH_4^+ breached ANZECC / ARMCANZ (2000) trigger levels (NO_x = 40 μg L^{-1}, NH_4^+ = 20 μg L^{-1}) 88 % of the time.

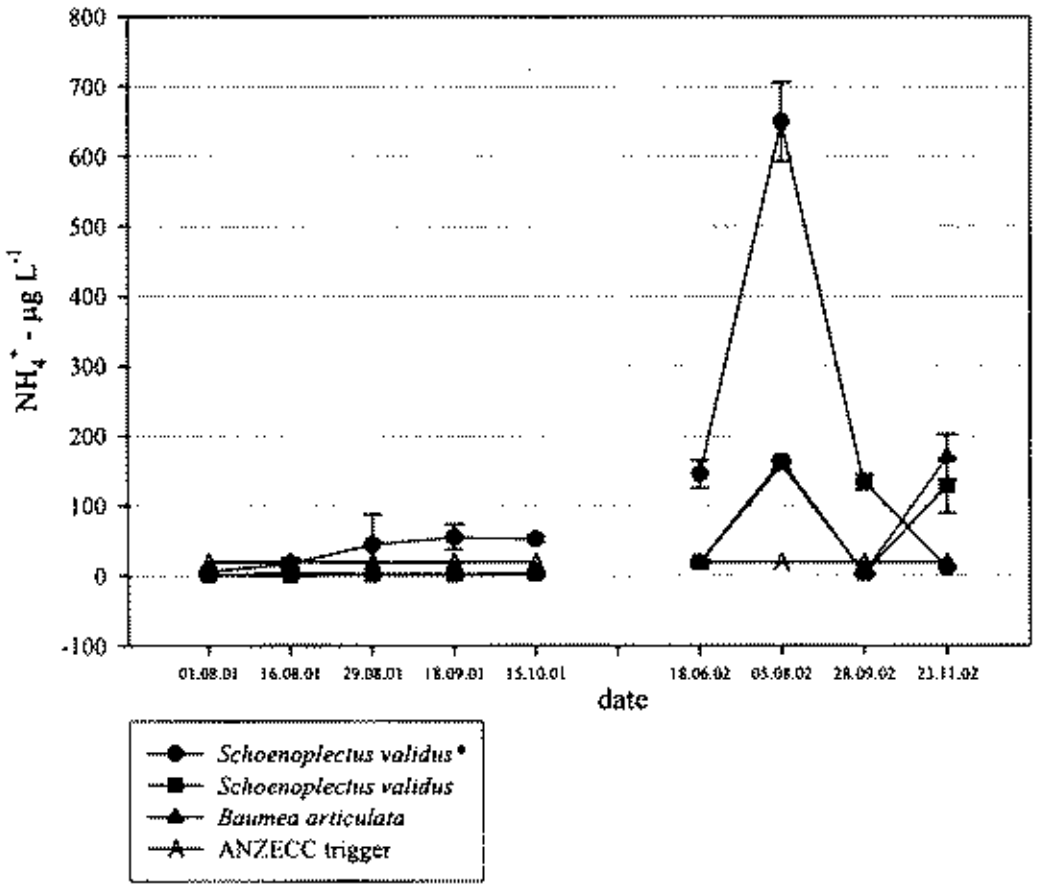


Figure 2.19. Wetland influent and effluent ammonium concentration. In this figure, culture wetland influents refer to tap water, and polishing wetlands influents refer to culture wetland effluents. * wetland R1-R2

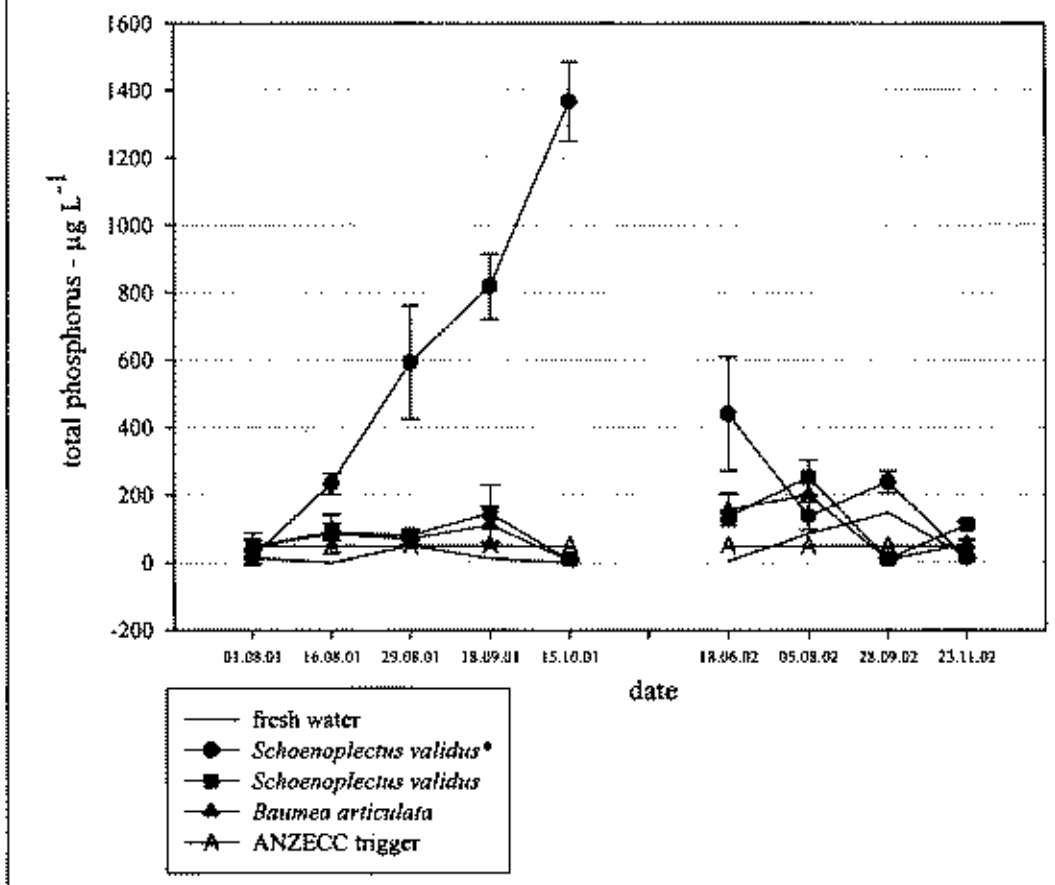


Figure 2.20. Wetland influent and effluent total phosphorus concentration. In this figure, culture wetland influents refer to tap water, and polishing wetlands influents refer to culture wetland effluents. * wetlands R1-R2

Culture wetland (R1-R2) effluent total phosphorus (TP) concentration ranged from 14 µg L^{-1} to 1.37 mg L^{-1} and averaged 428.8 µg L^{-1}, breeching the ANZECC / ARMCANZ (2000) trigger value 88 % of the time (Figure 2.20).

Filterable reactive phosphorus (FRP) ranged from 1 µg L^{-1} to 372 µg L^{-1} and averaged 71 µg L^{-1} (Figure 2.21), breaching the ANZECC / ARMCANZ (2000) trigger level (20 µg L^{-1}) 44 % of the time. Suspended solids remained below the ANZECC / ARMCANZ (2000) trigger level 100 % of the time with concentrations ranging from 0.3 mg L^{-1} to 1.4 mg L^{-1}, and averaging 1.1 mg L^{-1} (Figure 2.22). In

each case, culture wetland effluent nutrient levels peaked near the end of trial 1 fish culture; and began to fall near the end of trial 2 fish culture.

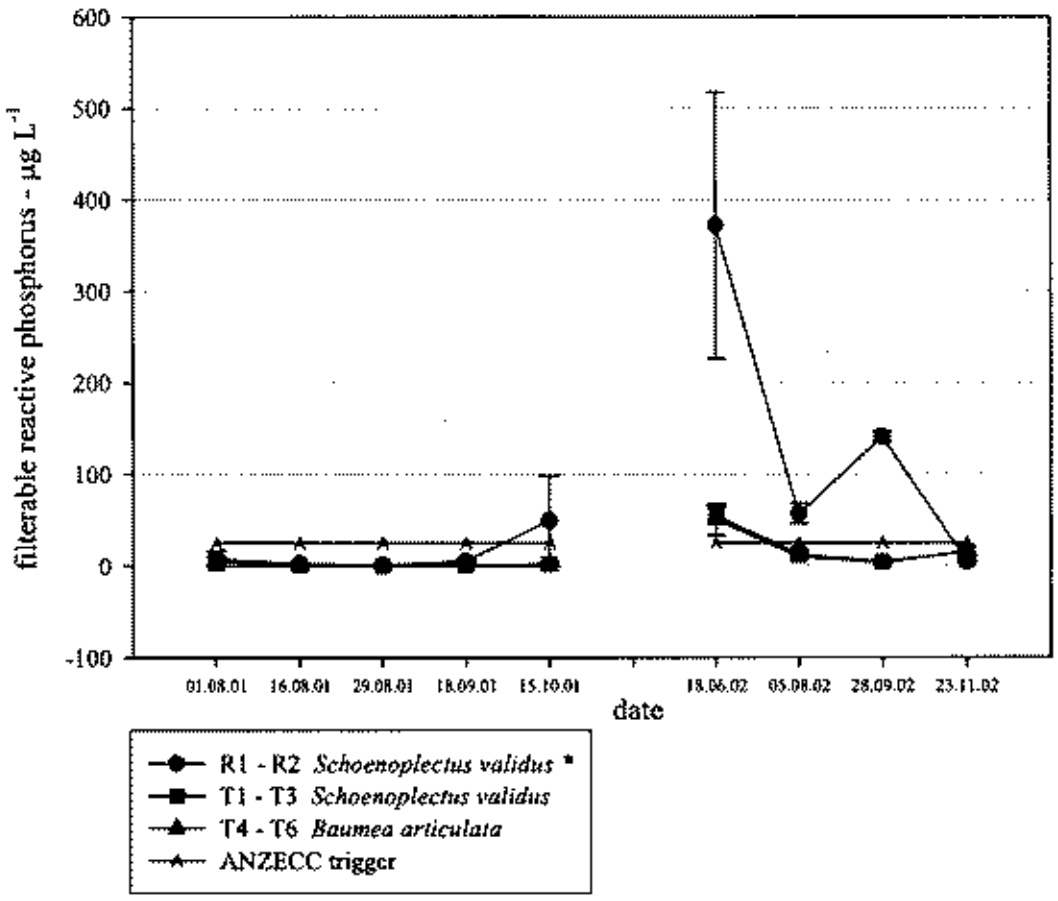


Figure 2.21. Wetland influent and effluent filterable reactive phosphorus concentration. In this figure, culture wetland influents refer to tap water, and polishing wetlands influents refer to culture wetland effluents.
* wetland R1-R2

Baumea articulata polishing wetland effluent TN concentration breached the ANZECC / ARMCANZ (2000) trigger level 44 % of the time by ranging 8 µg L^{-1} to 796 µg L^{-1}, and averaging 311 µg L^{-1} (Figure 2.17). Oxidized nitrogen concentrations ranged nil to 1.06 mg L^{-1}, averaging 166 µg L^{-1}; and ammonium ranged zero to 170 µg L^{-1}, averaging 40 µg L^{-1}. Both nitrogen configurations breached the ANZECC / ARMCANZ (2000) trigger level 33 % of the time (Figures 2.18-2.19). Wetland effluent TP concentration ranged 6 µg L^{-1} to 201 µg L^{-1} and averaged 83 µg L^{-1}, breeching the ANZECC / ARMCANZ (2000) trigger level 88 % of the time (Figure

2.20) while FRP ranged zero to 55 µg L^{-1}, averaging 11 µg L^{-1}, and breaching trigger levels 11 % of the time (Figure 2.21). Suspended solids concentrations did not breach trigger levels, and ranged 1.0 mg L^{-1} to 3.0 mg L^{-1}, averaging 2.0 mg L^{-1} (Figure 2.22).

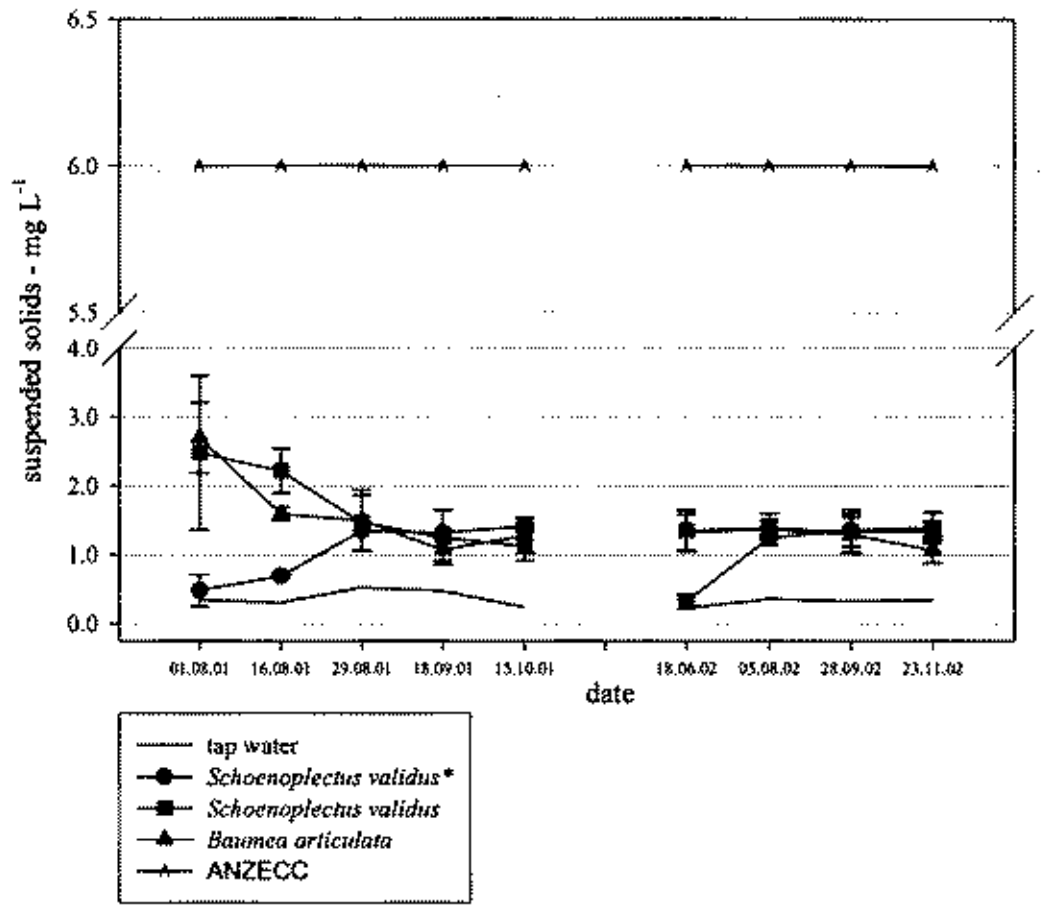


Figure 2.22. Wetland influent and effluent suspended solids concentration. In this figure, culture wetland influents refer to tap water, and polishing wetlands influents refer to culture wetland effluents. * wetland R1-R2

Schoenoplectus validus polishing wetland effluent TN, NO_x, and NH_4^+ breached the ANZECC / ARMCANZ (2000) trigger level 33 % of the time. Total nitrogen concentration ranged 12 μg L^{-1} to 940 μg L^{-1}, averaging 382 μg L^{-1} (Figure 2.17). Oxidized nitrogen concentrations ranged 6 μg L^{-1} to 463 μg L^{-1}, averaging 108 μg L^{-1}; and ammonium ranged nil to 164 μg L^{-1}, averaging 36 μg L^{-1} (Figures 2.18-2.19). *Schoenoplectus validus* polishing wetland effluent total phosphorus concentration ranged 8 μg L^{-1} to 252 μg L^{-1}, averaging 99 μg L^{-1}, and breached ANZECC / ARMCANZ (2000) trigger levels 88 % of the time (Figure 2.20) while filterable reactive phosphorus ranged zero to 51 μg L^{-1}, averaging 9 μg L^{-1}, and breached ANZECC / ARMCANZ (2000) trigger levels 11 % of the time (Figure 2.21). Suspended solids concentrations did not breach trigger levels, and ranged 1.1 mg L^{-1} to 2.5 mg L^{-1}, averaging 2 mg L^{-1} (Figure 2.22).

The effluent NO_x concentration in the *Baumea articulata* polishing wetland effluent was significantly lower ($P < .05$, $F_{1,4}$) than that of *Schoenoplectus* wetland effluent concentrations. The statistical difference was limited to the first batch-flow event in trial 2 (June 2002), where effluent NO_x concentrations in all wetlands were at their highest. There were no statistical differences between *Schoenoplectus validus* and *Baumea articulata treatments* with respect to effluent TN, NH_4^+, TP, FRP, and SS concentrations ($p > .05$)

Concentrations of total nitrogen, oxidized nitrogen, ammonium, total phosphorus, and filterable reactive phosphorus within polishing wetlands increased over time ($p < .01$) and suspended solids decreased ($p < .05$). Polishing wetlands removed 84 % of the TN, and 86 % of the TP entering in the form of polyculture wastewater inputs (Table 2.9) with removal efficiencies (i.e. effluent concentration /

influent concentration x 100) for *Baumea articulata* wetland marginally better than those for *Schoenoplectus validus*.

	% TN removal	% TP removal
T1	83.5	85.4
T2	84.4	85.3
T3	83.7	86.2
mean	83.9	85.6
stdv	0.5	0.5
T4	87.4	86.0
T5	83.6	89.1
T6	82.4	86.3
mean	84.4	87.1
stdv	2.6	1.7

Table 2.9. Polishing wetlands removal of nitrogen and phosphorus from polyculture wastewater and tap water inputs.

2.6.9 Hydrology

Data for trials 1 - 2 hydrology and water quality are consolidated and reported separately to the data for trial 3. This is because trial 3 did not involve the use of the polishing wetlands, and was not included in the nutrient water quality and system CNP mass balance calculations.

In trials 1 and 2 the culture wetland / fish tank sub-systems (R1-R2) used on average 38.5 m^3 of tap water in addition to 7.9 m^3 of rainfall received, totaling 46.4 m^3 water input over trials 1 and 2 (Figure 2.23). Mean output distribution of water inputs was partitioned into evapotranspiration (66%), wastewater discharge (23 %), and residual (i.e. left over in the wetland after the trials) wetland volume (8%), including a 2% error (Figure 2.24). The total volume of tap and rain water applied

to, and captured by, R1 - R2 in both trials totaled 92.7 m^3. The ratio of total water usage (m^3) to total fish production (kg) in trials 1 and 2 was 2.0 : 1.0, and in trial 3 the ratio of total water usage (m^3) to total fish production (kg) was 1.2 : 1.

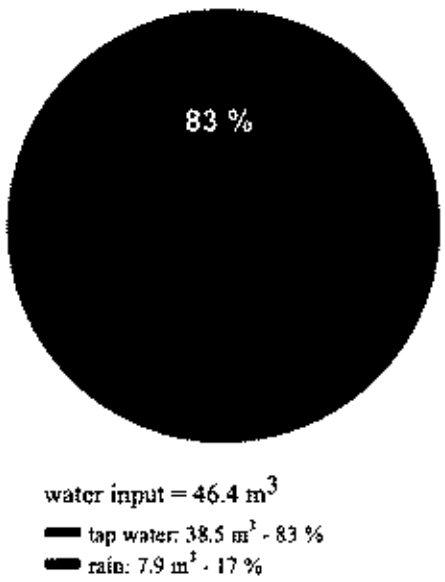


Figure 2.23. Origin of culture wetland (R1-R2) water for trials 1 and 2.

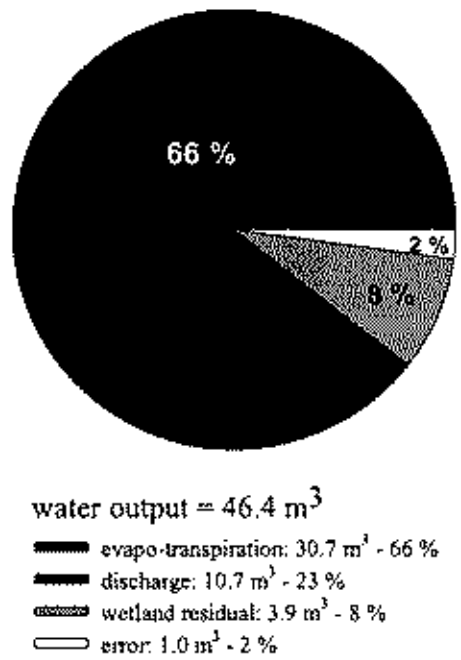


Figure 2.24. Culture wetland (R1-R2) water output distribution; evapo-transpiration, discharge wastewater, wetland residual, and error for trials 1 and 2.

Water input to polishing wetlands was controlled manually, and also fell into polishing wetlands as rain. There were no statistical differences between polishing wetland treatments with respect to partitioned output volumes ($p > .05$). *Baumea articulata* and *Schoenoplectus validus* replicates received 7.1 m^3 influent partitioned as R1-R2 system wastewater (2.9 m^3), rainwater (2.6 m^3), and tap water (1.6 m^3) sources (Figure 2.25).

Baumea articulata

ultimately distributed influent as evapotranspiration (59 %), wastewater discharge to evaporation basin (28 %), and residual wetland water (14 %), with 2 % error (Figure 2.26). *Schoenoplectus validus* ultimately distributed influent as evapotranspiration (58 %), wastewater discharge evaporation basin (29 %), and residual wetland water (15 %), with 2 % error (Figure 2.27).

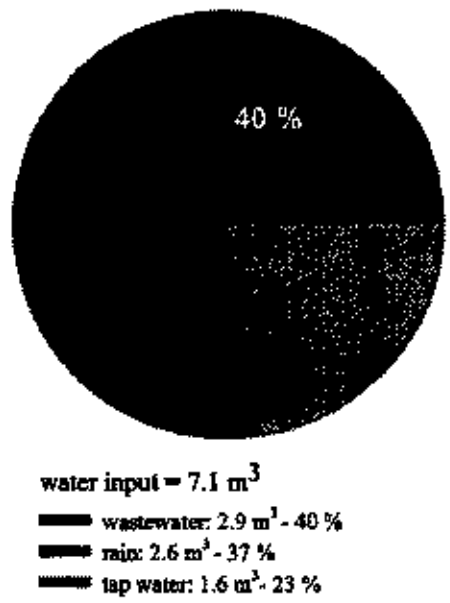


Figure 2.25. Origin and distribution of wetland T1-T6 wastewater influent, tap water, and rain for trials 1 and 2.

In trial 3 the culture wetland / fish tank sub-systems (R1-R2) used on average 29.1 m^3 tap water in addition to 1.8 m^3 of rainfall received, totaling 30.9 m^3 of water input (Figure 2.28). Terminal distribution of water input was partitioned into evapotranspiration (61%), wastewater discharge (24 %), and residual wetland water (10%), with 5 % error (Figure 2.29). The collective total volume of tap and rain water used by R1 - R2 in trial 3 equaled 58.1 m^3. The ratio of total water usage (m^3) to total fish production (kg) in trial 3 equaled 0.86 : 1.0.

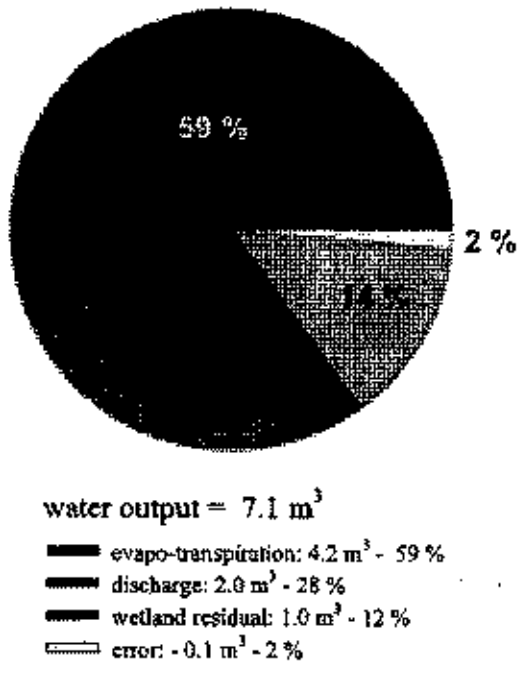


Figure 2.26. Wetlands T4-T6 (*Baumea articulata*) water output distribution; evapo-transpiration, wastewater discharge, wetland residual, and error for trials 1 and 2.

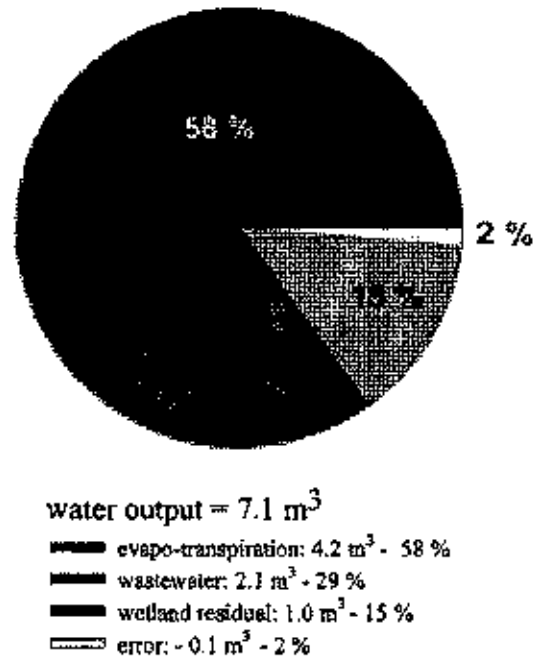


Figure 2.27. Wetlands T1-T3 (*Schoenoplectus validus*) water output distribution; evapo-transpiration, wastewater discharge, wetland residual, and error for trials 1 and 2.

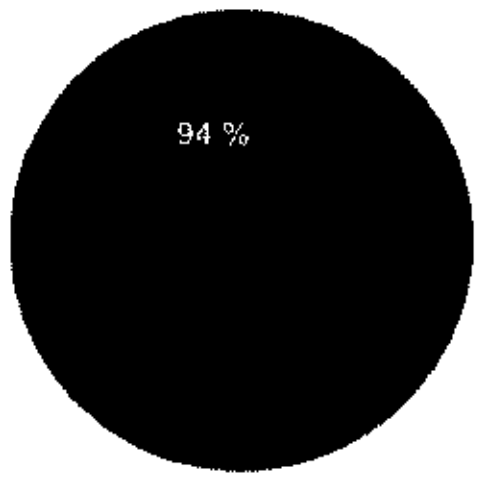


Figure 2.28. Culture wetlands input distribution of tap water and rain water for trial 3.

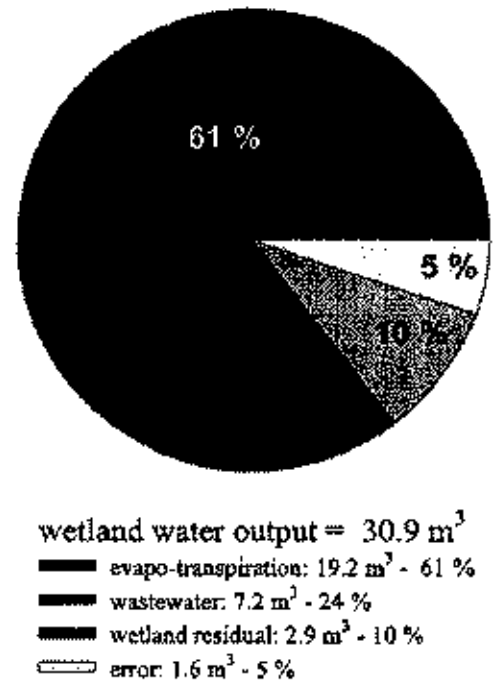


Figure 2.29. Culture wetlands water output distribution; evapotranspiration, discharge wastewater, wetland residual, and error for trial 3.

2.6.10 Carbon - nitrogen - phosphorus mass balance

When considering system inputs from a total system perspective (i.e. all fish tanks and wetlands), atmospheric carbon represented the greatest system input bound in plants via photosynthesis with 60.1 kg of carbon recovered in plant matter (figure 2.30).

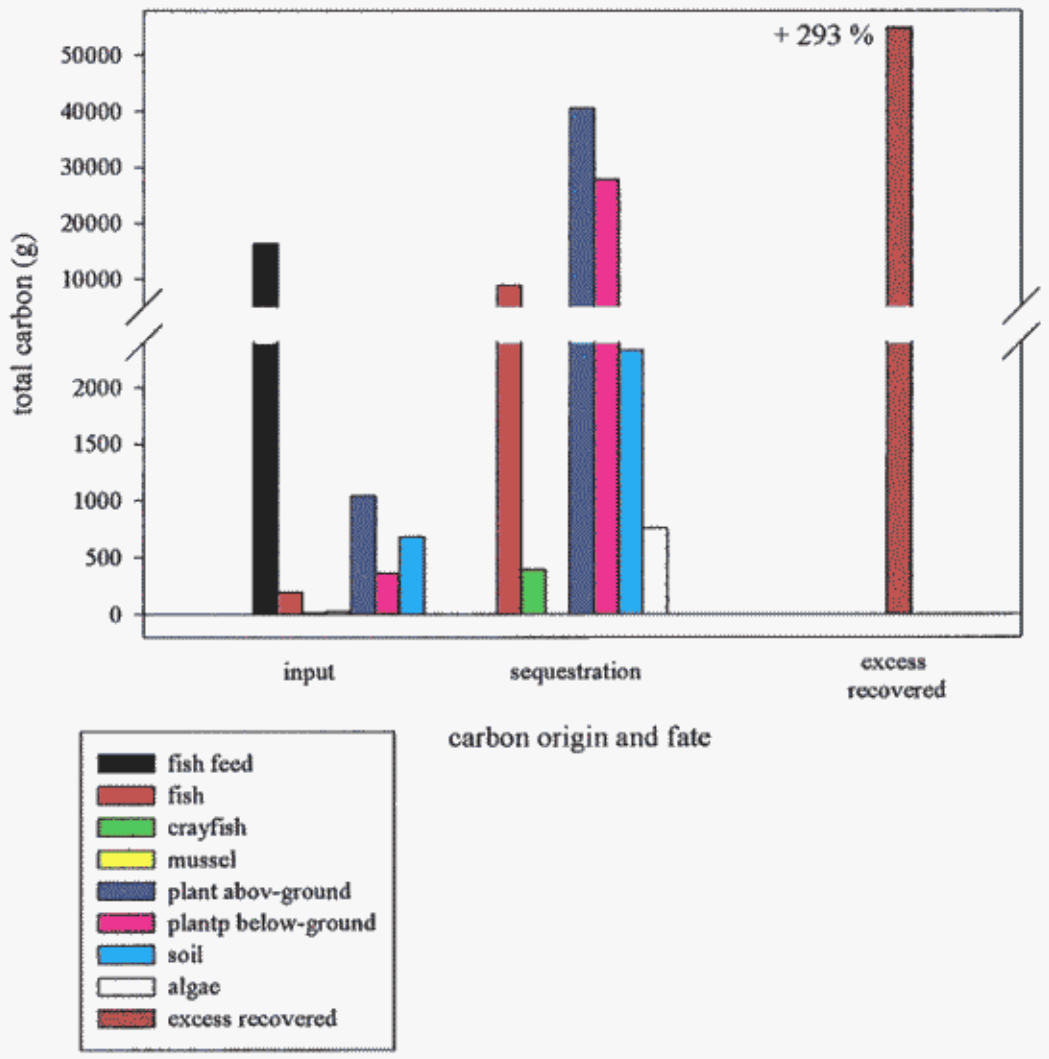


Figure 2.30. System carbon mass balance.

Note that in mass balance, bar graph figures "excess recovered" or "not recovered" categories represent the difference between total "inputs" and "sequestration / outputs" measured. For all practical purposes it is impossible to measure all the

nutrient inputs and outputs of an open ecosystem such as the integrated wetlands systems trialed in this experiment. For example, in the instance (Figure 2.30) one might expect an "excess recovery" of carbon because the total amount of atmospheric CO_2 entering the plants was not measured as a direct input, although it was measured sequestered as plant biomass at the time of wetland harvest.

Fish feed represented the 2nd largest carbon source, as well the largest source of both nitrogen and phosphorus input: 16.435 kg of carbon, 3.080 kg of nitrogen, and 0.513 kg of phosphorus entered the system as administered fish feed. Figures 2.30 - 2.32 depict fish feed CNP inputs within a system mass balance (i.e. the sum of all measured inputs and outputs analysis).

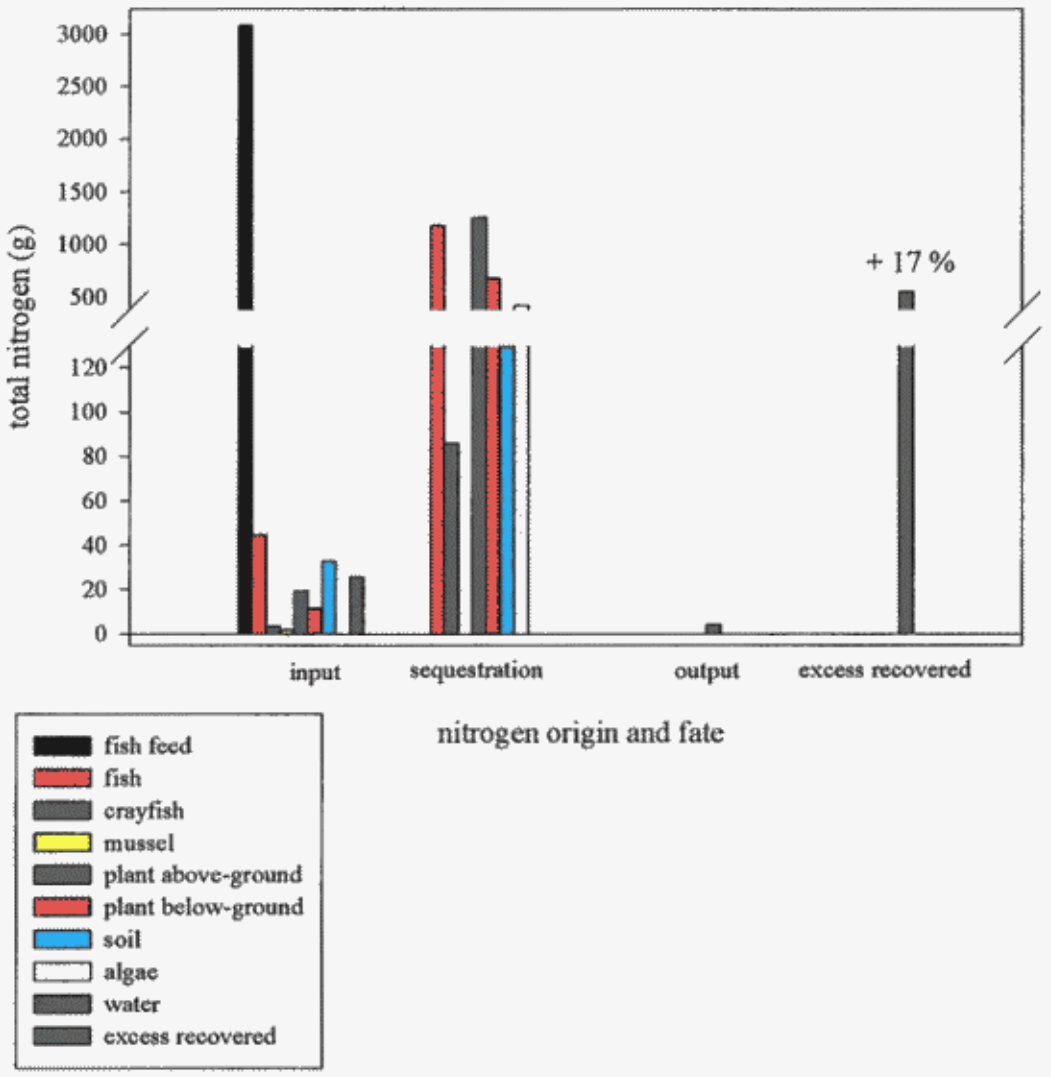


Figure 2.31. System nitrogen mass balance.

Lates calcarifer were able to convert 53 % of the carbon, 37% of the nitrogen, and 38 % of the phosphorus entering the system in the form of fish feed to fish biomass (Table 2.10). In comparison to other nutrient reservoirs, fish biomass sequestered the most phosphorus, the second greatest amount of nitrogen (intermediate between plant below ground and above ground biomass), and retained the most carbon of all non-photosynthetic reservoirs.

Cherax quadricarinatus retained the equivalent of 2 % of the carbon, 3 % of the nitrogen, and 3 % of the phosphorus entering the system as fish feed (Table 2.10). In comparison to the other nutrient reservoirs, crayfish sequestered the least amount of carbon and nitrogen, and ranked 2nd last in the mount of phosphorus retained, ahead of only algae (Figures 2.30 - 2.32).

For *Velesunio ambiguus* the only CNP inputs into the system occurred in the experiment due to total mortality. Figures 2.30 - 2.32 included mussel CNP inputs within the system mass balance.

Wetland substrates sequestered 10 % of the carbon, 3 % of the nitrogen, and 11 % of the phosphorus entering the system as fish feed (Table 2.10). In comparison to the other nutrient reservoirs, soil ranked 4th largest behind fish and plant above and below ground biomass in carbon and phosphorus retention, and 5th largest in the amount of nitrogen sequestered, ahead of only crayfish (Figures 2.30 - 2.32).

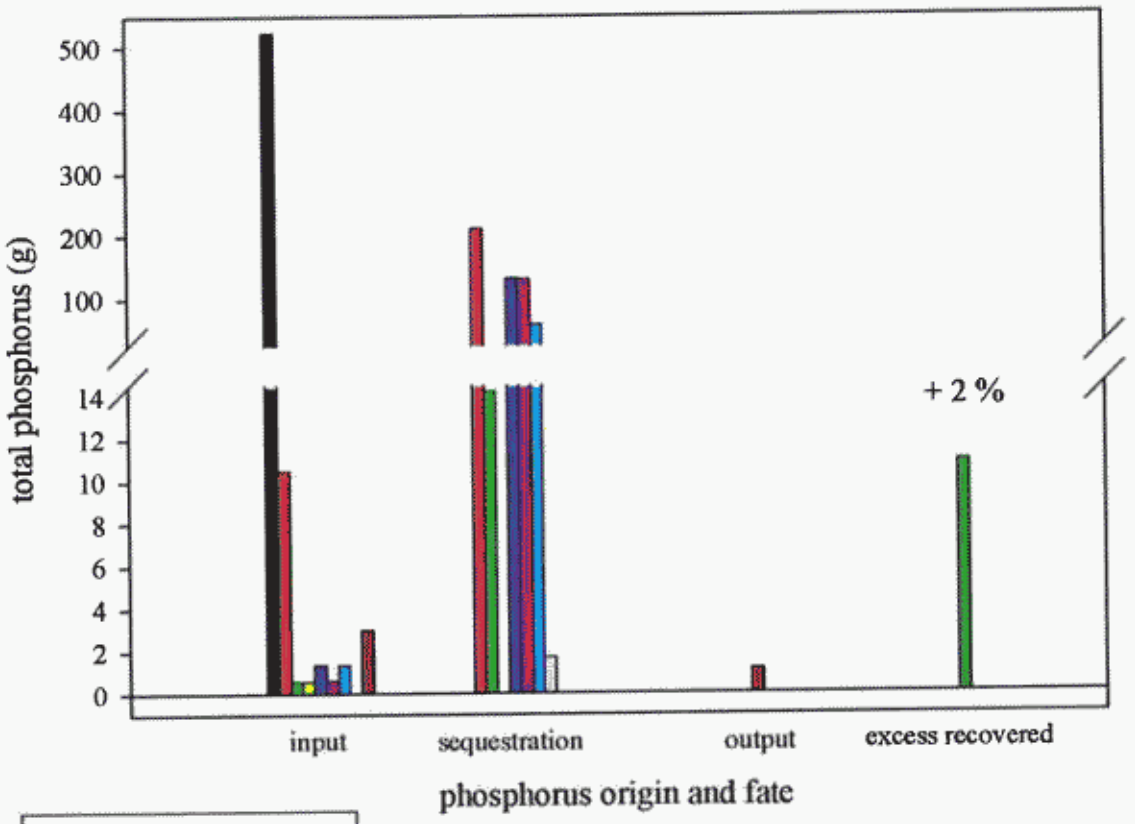


Figure 2.32. System phosphorus mass balance.

For wetland plants, comparatively plant above ground dry biomass sequestered an average of 243 % of the carbon, 41 % of the nitrogen, and 25 % of the phosphorus entering the system as fish feed. Plant below ground dry biomass sequestered comparatively an average of 123 % of the carbon, 22 % of the nitrogen, and 25 % of the phosphorus entering the system as fish feed (Table 2.10). Both above and below ground plant biomass ranked largest in carbon sequestration and 2nd largest in phosphorus sequestration (behind fish biomass). Plant above ground

biomass ranked 1st in nitrogen sequestration, with below ground biomass nitrogen ranking 3rd behind plant shoot and fish sequestration (Figures 2.30 - 2.32).

Wetland algae sequestered an average of 5 % of the carbon, 14 % of the nitrogen, and 0.3 % of the phosphorus entering the system as fish feed (Table 2.10). Algae sequestered the least amount of phosphorus. Nitrogen sequestration ranked 4th behind fish and plant biomass, and carbon sequestration ranked 5th surpassing only crayfish biomass retention (Figures 2.30 - 2.32). Algal recovery in the culture wetlands was restricted to soil fractions, as filamentous clumps or mats in the water column or on its surface were not present.

	% total carbon sequestered	% total nitrogen sequestered	% total phosphorus sequestered
fish	52.6	36.9	38.4
crayfish	2.3	2.7	2.6
plant shoot	242.6	40.6	25.2
plant root	122.8	21.5	25
algae	4.6	13.7	0.3
soil	10	3.1	11.1
wastewater	na	0.1	0.2

Table 2.10. System CNP sequestration and discharge. Data are % sequestered as a proportion of fish feed inputs.

For each kilogram of fish produced, 35.7 grams of TN and 16.6 g of TP were contained in the culture wetland effluents. After those effluents received polishing wetland treatment, they contained 2.1 g of TN and 0.6 g of TP per kg of fish produced.

In comparison to other nutrient sequestrators, a small fraction of feed input nitrogen and phosphorus were transported from the system as discharge water output

(Figures 2.30 – 2.32). Water discharged from the system retained on average 0.1 % of the nitrogen and 0.2 % of phosphorus entering the system as fish feed (Table 2.10).

Total system CNP mass balance (i.e. feed, soil, water, mussels, fish, crayfish, plants) resulted in excesses of 293 % carbon, 17 % nitrogen, and 2 % phosphorus recovered within system sequestration reservoir and output substrates (Table 2.11, Figures 2.30 - 2.32).

	% carbon	% nitrogen	% phosphorus
Complete System: R1-R2 & T1-T6	292.6	16.9	1.8
R1 - R2 *S. validus*	279.7	3.9	3.7
T1 - T3 *S. validus*	709	1460.5	- 4.6
T4 - T6 *B. articulata*	580.7	433.3	- 5.1

Table 2.11. System carbon, nitrogen, and phosphorus mass balance. Data are % CNP sequestered (including wastewater discharge) in respect to all nutrient inputs. An analyses of variance was not applied at the wetland level (see section 2.5 – paragraph 2); however anova analyses were applied at the substrate level.

Culture wetlands R1-R2 mass balance revealed excesses of 280 % carbon, 4 % nitrogen , and 4 % phosphorus (Table 2.11), with the hierarchical stratification of CNP within measured within culture wetland reservoirs reflecting that of the system mass balance (Figures 2.33 – 2.35). In comparison to polishing wetlands, culture wetland plants and soils were enriched with nitrogen and phosphorus (Table 2.34 – 2.35), undoubtedly due to the nutrient rich environment produced by the animal culture wastes; when dried and pulverized the enriched soil had a much darker brown color.

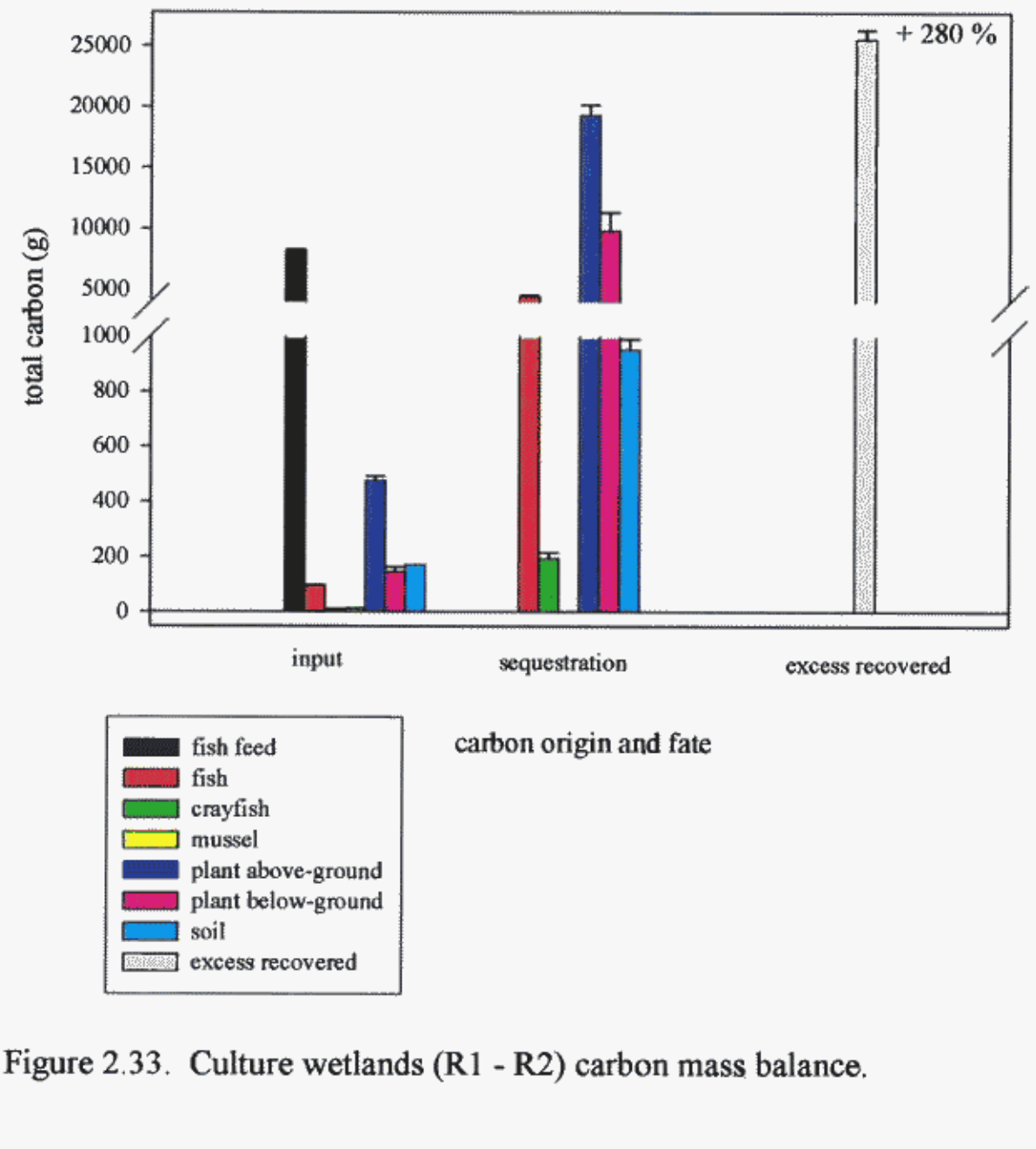


Figure 2.33. Culture wetlands (R1 - R2) carbon mass balance.

The CNP mass balance of *Baumea articulata* polishing wetlands revealed excesses of 581 % carbon, 433 % nitrogen, and a net loss of 5 % phosphorus (Table 2.11). Nitrogen and phosphorus inputs were dominated by water influents. Carbon analysis was not completed on water samples. Plant biomass sequestered the greatest amount of carbon and phosphorus, and nitrogen (Figures 2.36 - 2.38). A population of *Azolla* spp. was not active at the time, hence not recovered from the polishing wetlands. Polishing wetland *Baumea articulata* plant samples had statistically lower concentrations of nitrogen at harvest than did *Schoenoplectus validus* plant samples ($p < 0.01$).

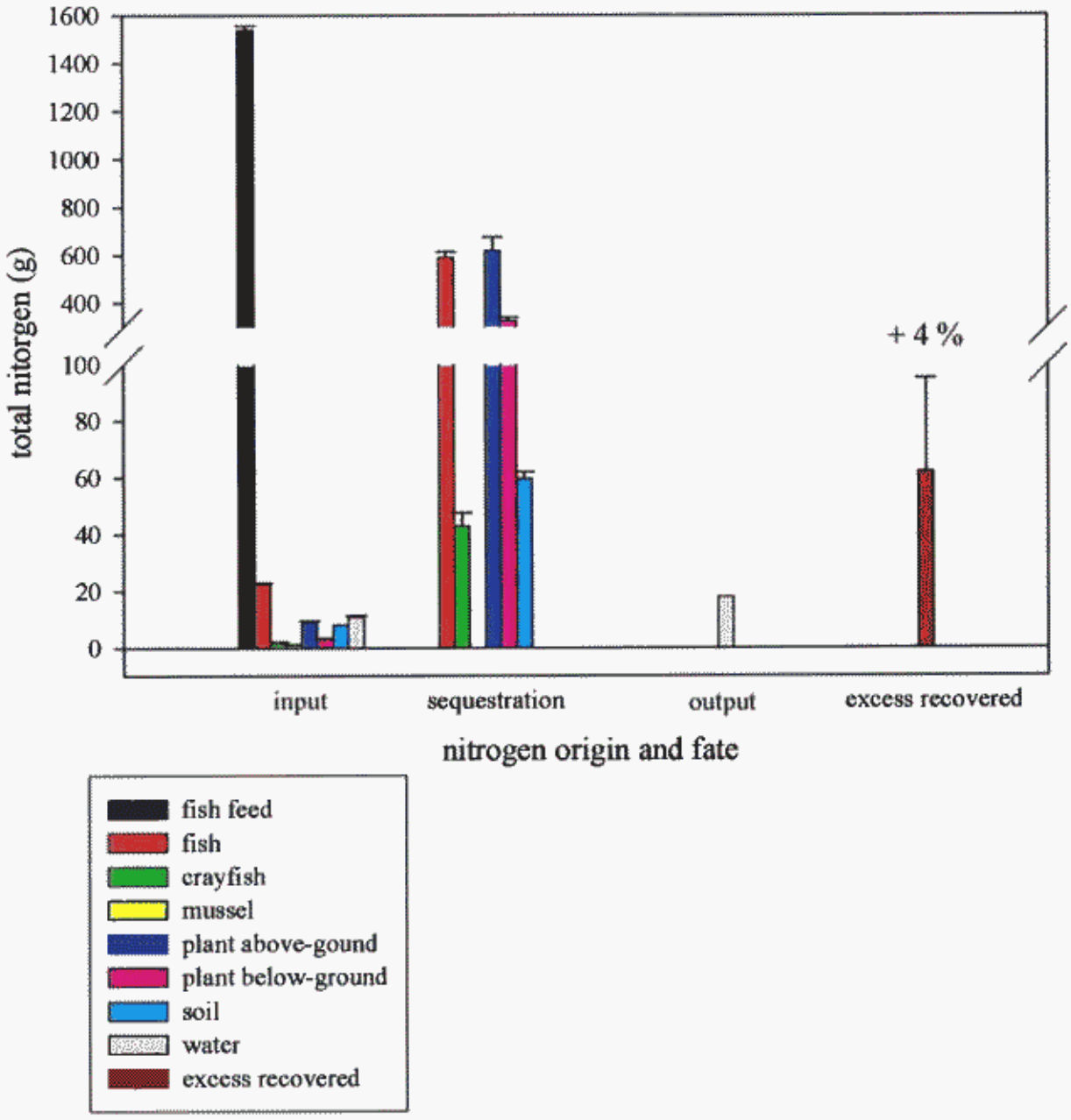


Figure 2.34. Culture wetlands (R1 - R2) nitrogen mass balance.

The CNP mass balance of *Schoenoplectus validus* polishing wetlands revealed excesses of 709 % carbon, 1 461 % nitrogen, and a net loss of 5 % phosphorus (Table 2.11). Nitrogen and phosphorus inputs were dominated by water influents, and in the case of nitrogen, fixation by algae and plants. Carbon analysis was not completed on water samples. Algae biomass sequestered the most CNP followed by above and below ground plant biomass, and then soil (Figures 2.39 – 2.41).

At the initiation of the experiment there were no differences in the amount of CNP in each polishing wetland in the form of plant, soil and water, or when substrate values were combined ($P > .05$).

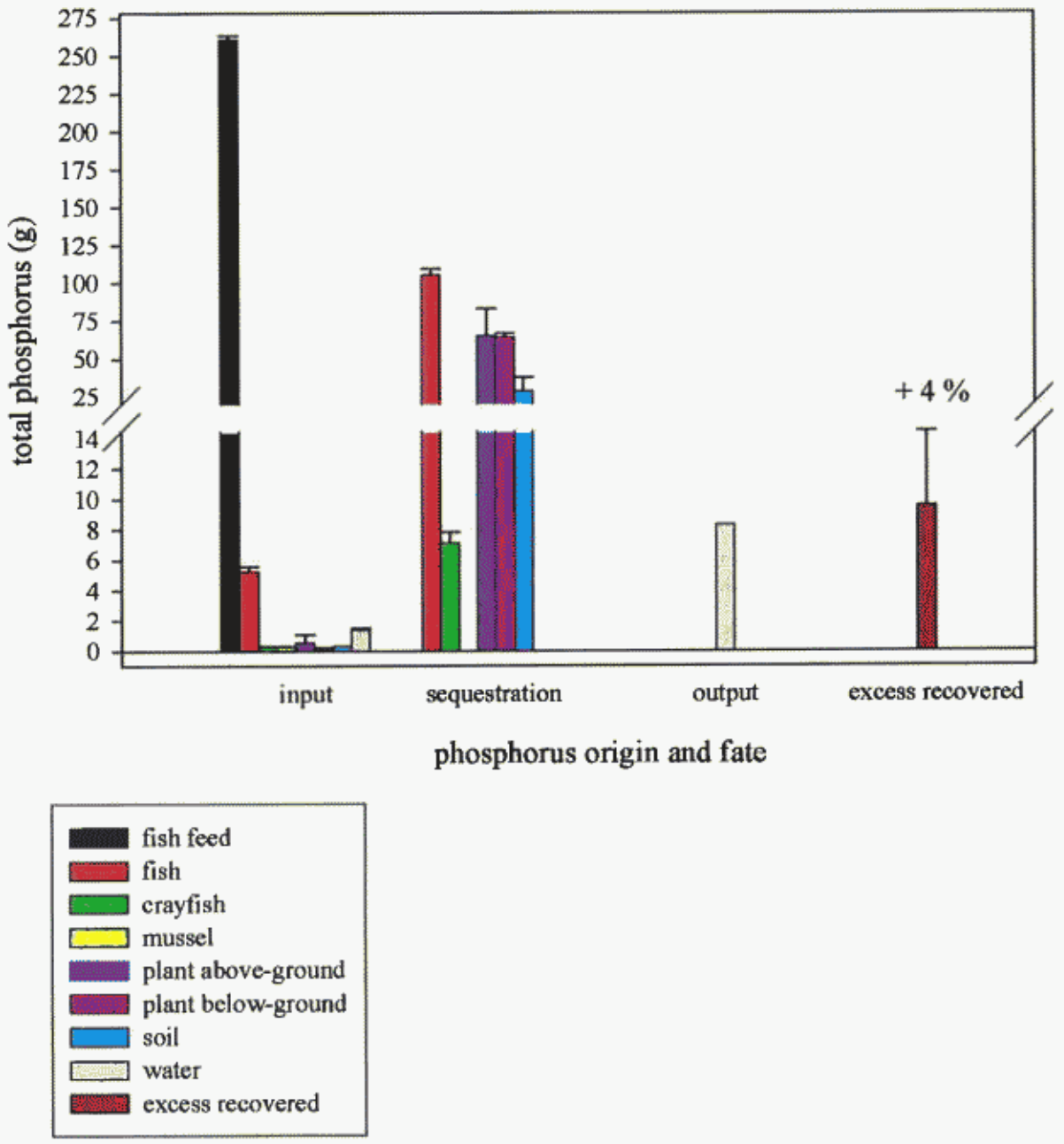


Figure 2.35. Culture wetlands (R1 - R2) phosphorus mass balance.

At the conclusion of the trials 1 and 2, the *Baumea articulata* wetlands sequestered more carbon in combined substrates (plant, algae, wastewater, and soil) than did *Schoenoplectus validus* wetlands ($P < .05$), and *Schoenoplectus validus* wetlands sequestered more nitrogen in combined substrates (plant, algae, wastewater,

and soil) than did *Baumea articulata* wetlands ($P < .05$). There was no difference in the amount of phosphorus sequestered in combined substrates between polishing treatments ($P > .05$).

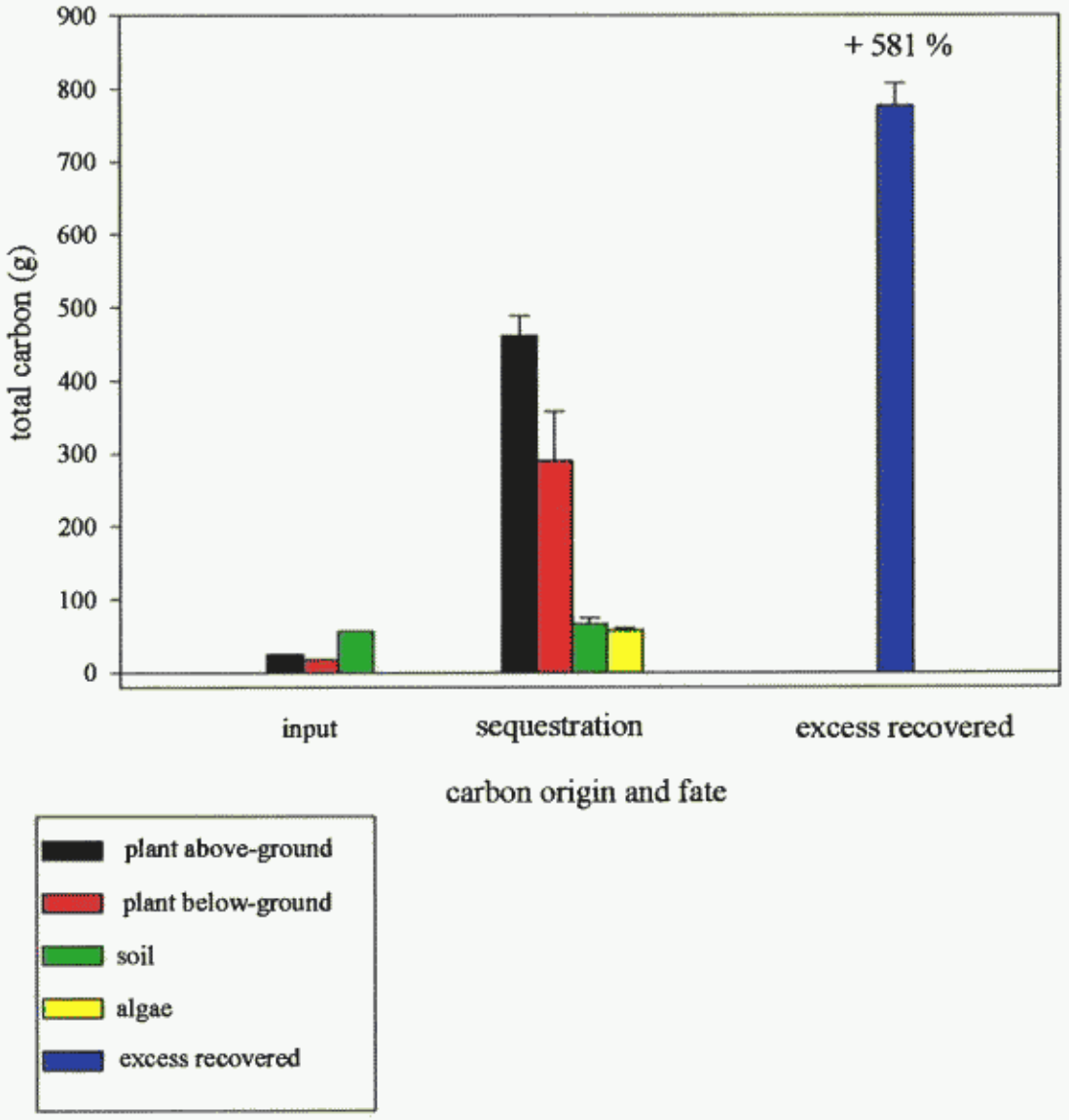


Figure 2.36. *Baumea articulata* wetlands carbon mass balance.

When comparing CNP recovery within individual substrates between polishing wetland treatments, *Baumea articulata* polishing wetlands recovered more carbon than did *Schoenoplectus validus* within plant biomass ($P < .05$), and *Schoenoplectus validus* polishing wetlands recovered more nitrogen than did *Baumea articulata* within algal biomass ($P < .05$).

When comparing CNP recovery within individual substrates between polishing wetland treatments, *Baumea articulata* polishing wetlands recovered more carbon than did *Schoenoplectus validus* within plant biomass ($P < .05$), and *Schoenoplectus validus* polishing wetlands recovered more nitrogen than did *Baumea articulata* within algal biomass ($P < .05$).

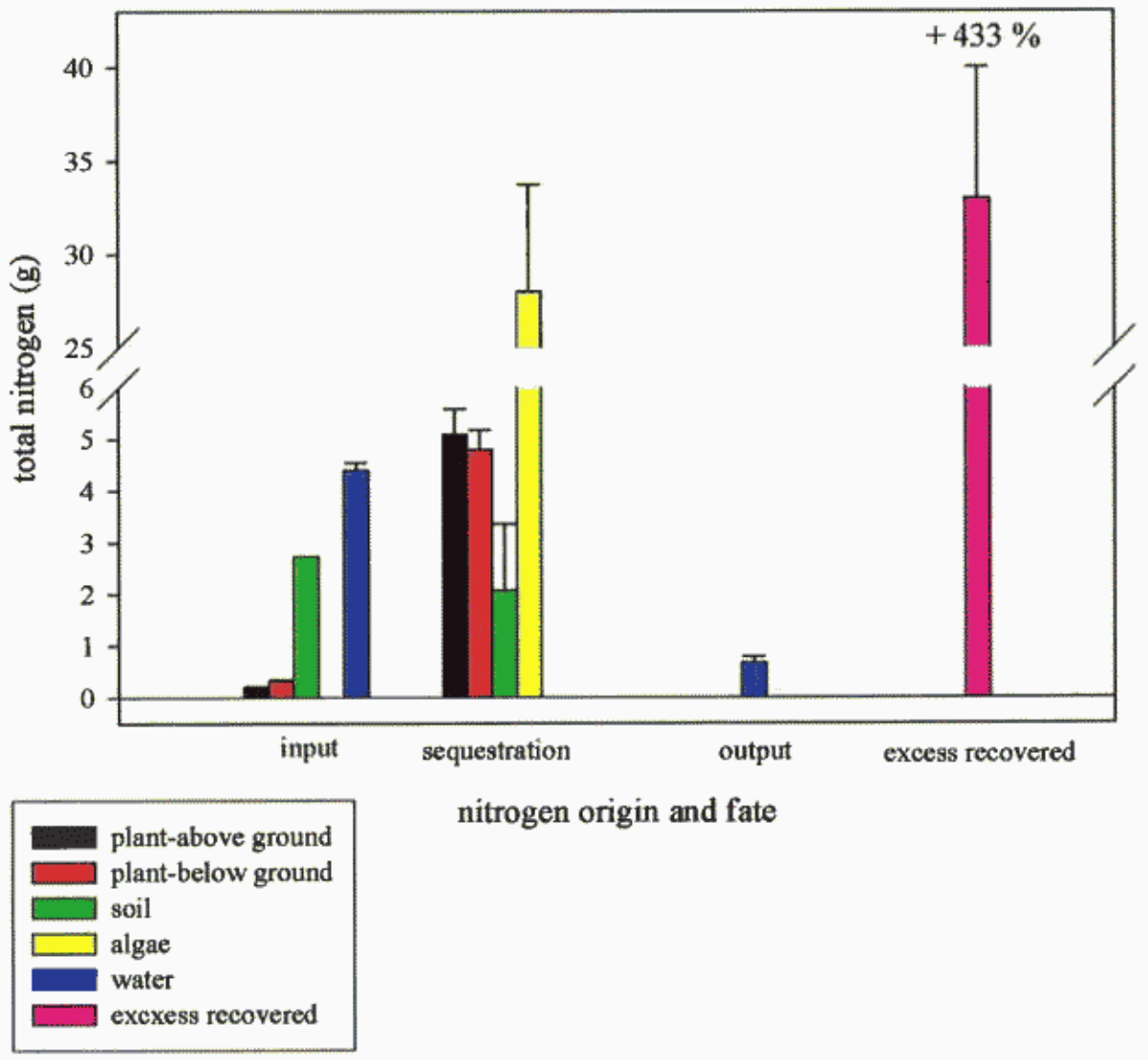


Figure 2.37. *Baumea articulata* wetlands nitrogen mass balance.

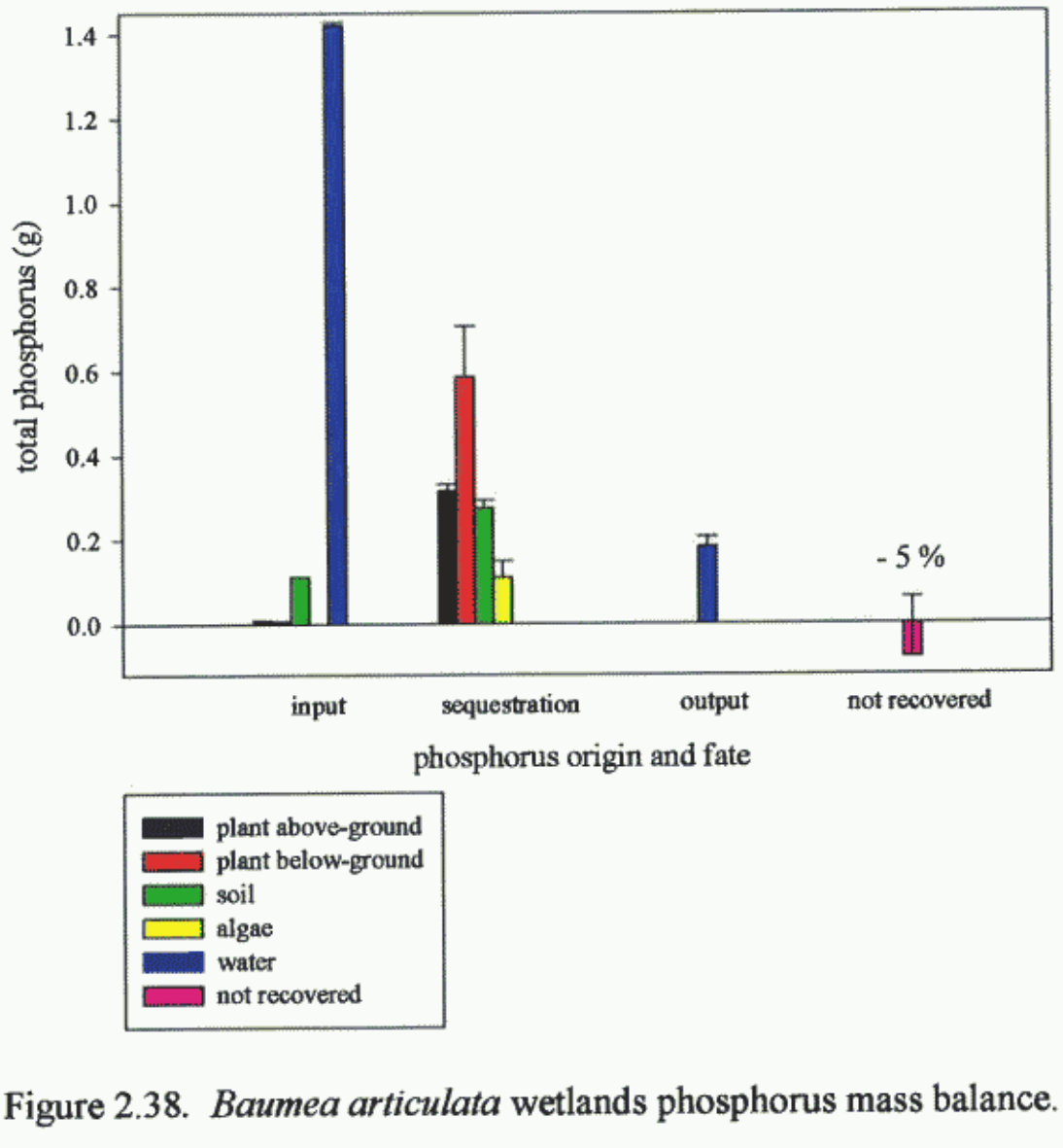


Figure 2.38. *Baumea articulata* wetlands phosphorus mass balance.

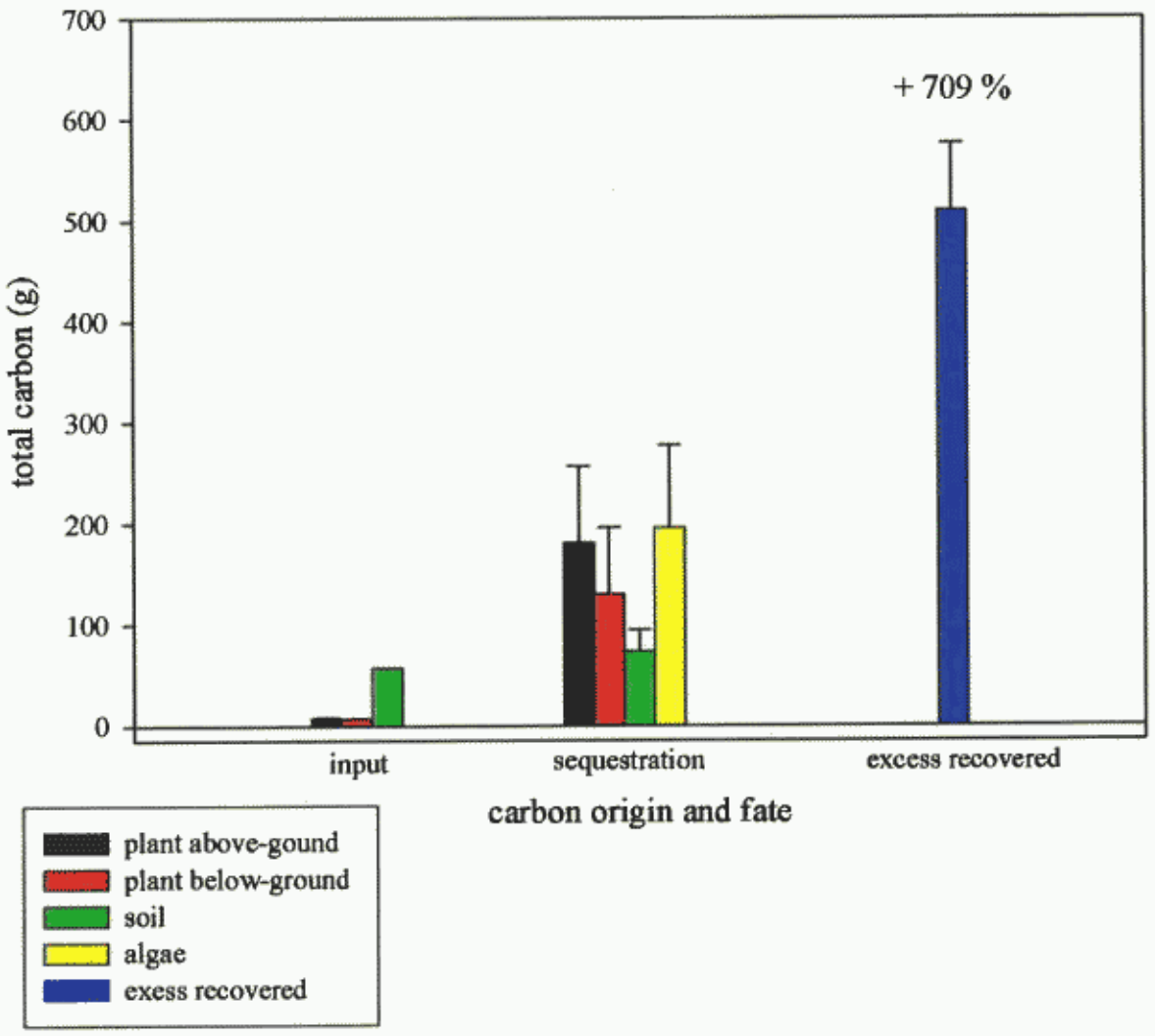


Figure 2.39. *Schoenoplectus validus* wetlands carbon mass balance.

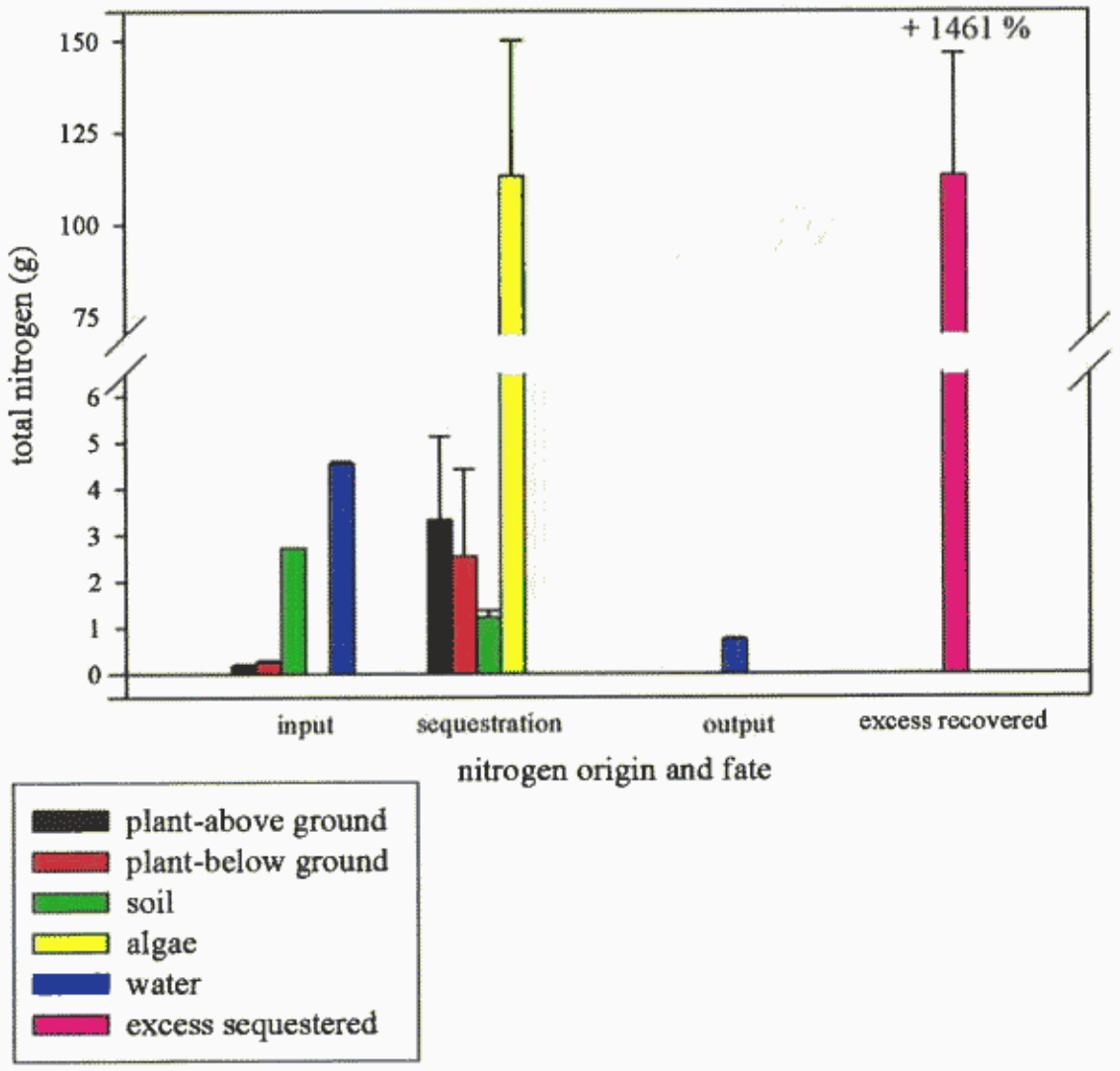


Figure 2.40. *Schoenoplectus validus* wetlands nitrogen mass balance.

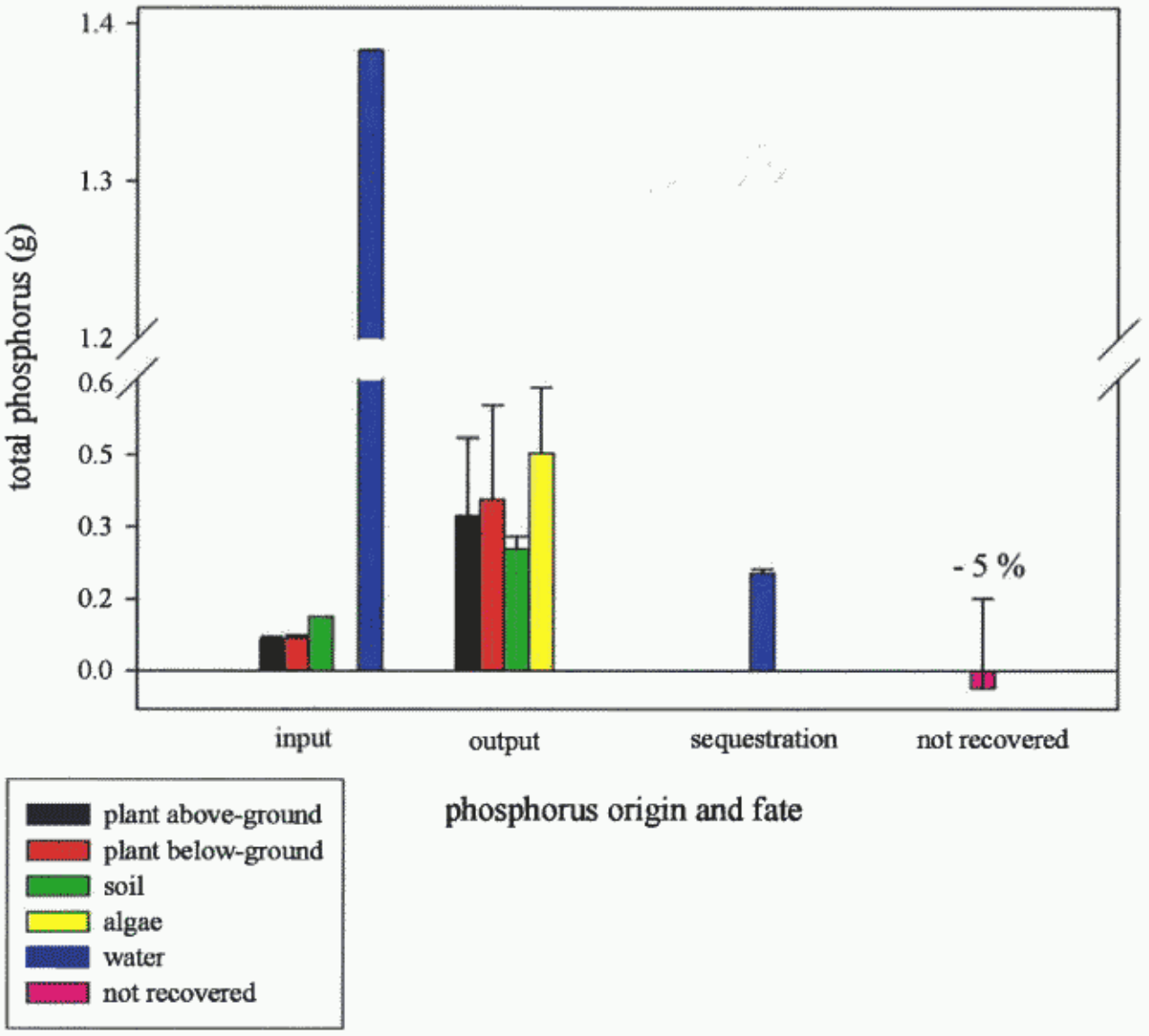


Figure 2.41. *Schoenoplectus validus* wetlands phosphorus mass balance.

2.7 Discussion

The integrated polyculture system was successful in many aspects not least in terms of the commercial-like culture of *Lates calcarifer* (see Section 2.7.1) and *Cherax quadricarinatus* (Section 2.7.2), wetland biodiversity support (see Sections 2.7.4 - 2.7.5), trialing of plant performance (Section 2.7.6), evaluation of water quality (Sections 2.7.7 - 2.7.8), hydrology (Section 2.7.9), and nutrient mass balance (Section 2.7.10). Tables 2.12 and 2.13 show comparative performance data from the fish and crayfish polyculture systems integrated wetland aquaculture systems outlined in sections 1.11 and 1.12, with the data from this experiment. Tables 2.12 and 2.13 are located at the end of this discussion section.

2.7.1 *Lates calcarifer*

Performance goals 1 and 2 were met in that mean fish specific growth rates were $\geq$ 1.5 % body weight d^{-1}, and fish feed conversion ratios were $\leq$ 1.5 : 1.0. In contrast, performance goal 3 was not met because as net water usage in trials 1 and 2 was twice the 1.0 m^3 per 1.0 kg fish harvest goal, although trial 3 went very close, requiring only 200 L of water per kg of fish production in excess of the performance goal 3 value.

The differences between trials 1 and 2 barramundi SGRs and FCRs were negligible. The moderate disparity between trials 1 and 2, and trial 3 were attributed to the variation between years in temperature and fish sizes at the time of stocking and harvest, with emphasis on the differences in seasonal temperature trends between the summer (trials 1 and 2) and winter (trial 3) crops. Additionally, it is

suggested the higher fish densities of trial 3 (nearly twice that of trial 1, and over 3 times that of trial 2) negatively impacted on the SGR and FCR through crowding and/or deterioration of water quality.

Excepting to this dissertation, there have been no reports of barramundi grown in integrated systems of any kind. The specific growth rates of barramundi cultured in trials 1 - 3 were at the very high end of published SRGs for barramundi monocultured in ponds or cages (Awang, 1987, Schipp, 1996) and for other fish species cultured in integrated wetland systems (Brummett and Alon, 1994, Costa-Pierce, 1998) (Tables 2.12 - 2.13). Comparisons with fish grown in polyculture with crayfish were not made as in those publications, for growth rates were reported as g d^{-1}, and not SGR.

The feeding rate (to satiation) of barramundi grown in trials 1 - 3 was near the lower end of published feeding rates for fish cultured in integrated wetland systems and for polyculture systems (Brummett and Alon, 1994, Costa-Pierce, 1998, Rouse and Kahn, 1998) (Table 2.12 – 2.13). The food conversion ratios of barramundi grown in trials 1 - 3 were very efficient when compared to published FCR values for barramundi monoculture (Lobegeiger, 2001, Barlow, 1996, Williams, 1996, Rimmer, 1995, Williams, 2003, Chaitanawisuti, 1994, Williams, 2000, Awang, 1987, Schipp, 1996), for other fish culture in integrated wetland systems (Brummett and Alon, 1994, Costa-Pierce, 1998), and for fish in polyculture systems (Karplus et al., 2001, Rouse and Kahn, 1998). The low FCRs measured in this experiment (e.g. ≤ 1.0) generally are restricted in the scientific literature to experimental or lab-scale systems. Thus the current system, in which fish were allowed to eat twice daily to satiation, was very efficient and almost negated the need

to re-adjust fish feeding calculations to efficiently meet anticipated requirements over the culture period as necessitated in similarly designed and managed systems.

Maximum barramundi density in respect to culture wetland and combined culture and polishing wetland surface area was at least 6 (culture) to 13 (combined) times greater than fish culture densities reported in the literature for all other integrated wetland systems, and was within the range of densities (fish per pond surface area) reported for tilapia polyculture with red claw (see Tables 2.11-2.12). The mass of fish produced over the 144 day period of trial 3 (kg fish per m^2 culture wetland surface area) approximated the estimated annual fish production (kg fish per culture pond surface area m^2) required by a barramundi farm if it was likely to be profitable in Queensland (Barlow, 1998). Consequently, this study illustrated the commercial viability of the integrated culture technique would not be restricted by limitations of fish culture density. Barring the pump failure in trial 3, survival of barramundi grown in trials 1 - 3 was what could be expected from successful commercial barramundi culture (Chaitanawisuti and Piyatiratitivorakul, 1994), and valued at the high end of fish survival reported in the literature for other integrated culture systems (Tables 2.12 – 2.13).

Parasite infections did not appear to impact on growth of fish. However, the *Epistylididae rhabdostyla* (gill parasite) infection in trial 3 would most likely have impacted on the growth of fish if allowed to persist (i.e. had not the fish crop been due for harvest), requiring a disinfesting salt bath, a standard and cheap method used by fish culturists to cleanse fish of freshwater parasites. Although not a problem encountered in this experiment, parasitic disease represented one possible draw back to using free-surface wetlands and recirculated water with aquaculture tanks in that

parasites could possibly establish resident populations within the wetlands, and be difficult to control or eradicate without damaging the ecosystem. However, it is equally likely that the complex trophic structures and abiotic / biotic aspects of a wetland ecosystem could afford resilience to the proliferation of a parasite community via competition and aquatic climate whereas, in a less complex system (e.g. managed fish pond), the parasites might grow and spread unchecked.

The overall highly efficient growth of fish in this experiment seemed due primarily to a combination of high physical, chemical and biological water quality maintained within the wetland system with an average temperature near 27 ° C, and regular hand feeding with quality commercial pellets in water. From biological and production efficiency standpoints, using low-tech, free-surface constructed wetlands as in-line water quality control for barramundi farming proved to be a technically viable, ecologically reasonable, alternative method of water quality control; despite the need for mechanical aeration for gross fish production to meet commercial viability values. Additionally, using wetlands in aquaculture may enhance the 'green' profile of the product thus increasing its market value.

2.7.2 *Cherax quadricarinatus*

The differences in red claw SGRs between trials 1 - 3 were negligible despite temperature decreases of *c.* 2 ° in successive trials. This apparent ability of the crayfish to maintain similar SGRs in the face of declining temperatures is explained as follows: in trial 2 the crayfish were at an earlier growth stage (smaller) than in trials 1 and 3, therefore had a naturally higher SGR (Jones, 1990) whereas in the trial

3, fish culture density was at its highest and the increased fish wastes indirectly provided more food to the resident populations of crayfish (detrital feeders) in the wetlands. Additionally, in both trials 2 and 3 mixed-sex populations were cultured in contrast to trial 1, in which only female crayfish were stocked in the wetlands. Male red claw have been shown to exhibit higher growth rates than females in pond culture systems (Sagi et al., 1997) and in this system may have co-buffered the effect of lower temperatures on SGR in trials 2 and 3.

The highest culture density of red claw was maintained in trial 2, and was the result of proliferation of trial 1 stock, the proliferation resulting from mistaken sexing and/or hermaphroditism/sex change. Female mono-sex proliferation was reported by Millin (1995) in experiments where hermaphroditism frequency was found to be 1.33 % of the female population: Millin also noted the difficulty in determining whether red claw were functional hermaphrodites or were in a transitional phase between sexes. Millin (1995) references two other sources that reported hermaphroditism rates of 4 % and 17 % and Kelley (1998) also reported sexual dimorphism in mono-sex cultured red claw (Kelley, 1998).

Even though crayfish in trial 2 were much smaller than those of trials 1 and 3, which provided them a metabolic advantage in terms of SGR, trial 2 SGR surprisingly was not greater and therefore the crayfish in trial 2 were almost certainly affected by the lower water temperatures acting in concert with correspondingly lower fish and higher crayfish densities. Natural wetland food eaten by crayfish was probably more limited in trial 2 (in this experiment, red claw were dependent solely on wetland primary production supported by fish culture waste and sunlight). It should be noted that in the early morning and late afternoon red claw were

commonly observed eating the aquatic plant *Azolla* sp. that resided transiently in the wetlands. Unequal periodicity of spawning between wetland R1 and R2 in trial 1 was thought to have caused the significant difference in individual red claw weights between the wetlands at the time of trial 2 harvest.

The specific growth rates of red claw in trials 1 - 3 were at the low end (less efficient end) of published SGR values for direct-fed pond cultured red claw (Pinto, 1996 , Jones, 1995, Jones, 1990, Rouse, 1998, Pinto, 1992, Austin, 1992). Survival of red claw in trials 1 - 3 was well within the range of published values (Rouse and Kahn, 1998, Pinto and Rouse, 1996, Jones, 1995).

Red claw growth in this experiment was much faster than the crayfish polycultured with tilapia in ponds invaded by wetland plants (Brummett and Alon, 1994) while maximum red claw culture densities were comparable in trial 1, but *c.* 2 and 3 times greater in trials 3 and 2, respectively. In each trial, survival was greater than was reported in the scientific literature for red claw in integrated wetland / red claw, and fish - red claw polyculture systems outlined in tables 2.12 and 2.13.

Red claw growth and survival in trials 1 and 3 were at the high end (more efficient) of published growth rates where red claw had been polycultured with fish in non-wetland (pond) systems (Karplus et al., 2001, Karplus et al., 1995, Rouse and Kahn, 1998), but trial 2 crayfish were on the lower end of the same efficiency scale. The maximum culture density of crayfish grown in trials 1 - 3 was similar to those reported in other polyculture systems (Karplus et al., 2001, Karplus et al., 1995, Rouse and Kahn, 1998)(Table 2.12 – 2.13).

The overall efficient growth of crayfish in this experiment most likely was

due to the biotic and abiotic characteristics of wetland system in this study and to water temperatures remaining within the published acceptable range for red claw. In addition to high physical, chemical and biological water quality, the wetlands provided custodial food and shelter niches in which red claw were able to grow and proliferate without additional resource inputs. Furthermore, crayfish grown in this system are grown from organic conversion of fish wastes, much like an organic cucumber would be grown using bat guano or pig manure soil amendment. Thus, the market value of crayfish products could potentially be enhanced by using this natural method.

Aquatic, avian, and mammalian predators were physically excluded or discouraged from access to the crayfish by the combined effect of 1.2 m poly walls (around a 10.75 m^2 area), pvc pipe shelters available to the red claw, thick plant growth, and tea-colored turbulent water. Predators were never witnessed foraging in the wetland integrated poly-culture system. However, blue-winged kookaburras and black cormorants commonly were observed feeding from other CQU red claw and fish ponds located adjacent to but apart from the system in this experiment. Additionally, cane toads (*Bufo marinus*) were observed breeding in dense numbers in the CQU pond system, and many cane toad tadpoles were observed within the CQU ponds.

Bi-annual red claw harvests would be necessary to control the density of crayfish in prolonged fish culture operations to avoid likely impacts of uncontrolled proliferation of crayfish on the wetland ecosystem (e.g. plant destruction and low water quality) and on the secondary crop itself (e.g. low population crayfish SGR,

and cannibalism). If, for whatever reason, at the time of harvest crayfish were too small to be marketed they could be fed back to the barramundi or sold as pond stock to other crayfish farmers.

From biological and production efficiency perspectives, successful growth and breeding of red claw in free-surface constructed wetlands that were used primarily as in-line water quality control for barramundi farming was shown to be possible with minimal management and no added food or heat inputs. However, it is pointed out that, without direct feeding, red claw grown in similar systems are unlikely to match the SGRs of red claw grown using direct-feed methods.

2.7.3 *Velesunio ambiguus*

Freshwater mussels were unable to persist in the wetlands over the duration of trials 1 and 2 suffering mortality from starvation. However, until death, mussels were observed to filter the water of plankton and other suspended particles. Thus, the mussels in this system were viewed as short term water filters. When the mussels died, the red claw scavenged the mussel bodies (preventing water fouling) leaving the clean empty shells which then became part of the wetland substrate, most likely leaching valuable calcium into the system.

2.7.4 *Litoria fallax*

Frogs thrived in the wetlands, attracted to the apparently permanent source of water and food offered free of *Bufo marinus* (cane toad) competition and less exposed to other predator species. In this system frog density appeared to be dependent upon the wetland canopy biomass, and most likely related to the number of available perching points. Although not quantified in this experiment, frogs in all life stages undoubtedly played a role in the wetland trophic structures. The wetlands successfully supported and enhanced the frog populations within the confines of the experiment, an impact that was extended across the constructed systems boundaries, and supporting existing regional biodiversity especially at the higher trophic levels (e.g. birds and reptiles) outside the experimental area.

While there are limited reports in the published scientific literature of biodiversity associated with other integrated systems, it appears that birds and frogs are most often noted (Table 2.12 – 2.13).

2.7.5 Wetland benthic macroinvertebrates

Wetlands successfully supported a range of benthic macroinvertebrates but dominated by the benthic, dragon and damsel flies, other fly larva, and snails - a result of wetland design. Colonization of the constructed wetlands was more difficult for aquatic species that were flightless, were not able to disperse effectively, or could not traverse the wetland walls easily, and so enter the constructed wetlands. Snails most likely entered the system attached to the plants as eggs or small adults, and/or crawling over the wetland walls from adjacent creek areas. Although it was

not quantified directly, snails, damselflies, and dragonflies are undoubtedly were part of the trophic structure of the wetlands in this experiment facilitating nutrient cycling within the system, and the movement of mass (e.g. carbon, nitrogen, and phosphorus) to and fro, across system boundaries. For instance, emerging flying insects were observed to be eaten by frogs and spiders perched above the waters surface, and presumably by other predators encountered as the transient insects traveled outside the experimental wetlands to the wider natural environment. Additional organisms spotted as regular users of the wetlands for food, water, shelter, and/or proliferation were terrestrial spiders, honey bees (dense numbers), hornets, wasps, adult dragonflies, grasshoppers, ants, small birds (swallows), *Litoria caerulea* (nocturnal frog), geckos (nocturnal lizard), and numerous other insects of a wide variety of species. Hence, the integrated system supported regional biodiversity, support that extended across the physical boundaries of the integrated system.

2.7.6 *Baumea articulata* and *Schoenoplectus validus*

In this experiment the null hypothesis of polishing wetland macrophyte species comparison (see Section 2.1.3) was rejected in terms of emergent plant biomass, canopy surface area, light penetration, and photosynthetic rate since *Baumea articulata* was superior for each attribute measured.

The faster relative growth rate, greater relative rate of light interception, and greater photosynthetic rate of polishing wetland *Baumea articulata* in comparison to polishing wetland *Schoenoplectus validus* plants resulted in a larger crop (in terms of biomass) harvested from polishing wetlands T4-T6. The results were indicative of

the ability of *Baumea articulata* to survive better in the low-available-nutrient conditions of the wetlands receiving secondarily treated polyculture wastewater effluent from a wetland integrated polyculture system.

Contrary to this though, the relative surface area expansion rate (RSR) of *Baumea articulata* plants was 0.03 less than that of *Schoenoplectus validus* (see section 2.6.6). Considering the statistically greater amount of *Baumea articulata* plant surface area measured at harvest, this discrepancy best explained as inadequate sensitivity (too coarse for a ± 0.03 sensitivity) of the methods used to measure the surface area of wetland plants at the time of planting. Additionally, at the time of planting, *Baumea articulata* plants did have more surface area than plants *Schoenoplectus validus*, contributing in a small way to the weakened effectiveness of the measurement at ± 0.03 precision. Because plant damage was not an issue during harvest, surface area measurements were made using more direct and precise methods at harvest.

Articles in the published literature reporting on the relative growth rate (RGR), relative surface area expansion rate (RSR), relative light penetration decrease rate (RLDR), or net photosynthetic rate of *Baumea articulata* or *Schoenoplectus validus* are not available. However, Keddy (1998) published a table that listed the relative growth rates of 48 wetland plant species. The slowest growing species in that table was *Eriocaulon septangulare* (a stress tolerator) with a RGR of 0.01. It is important to note that the plant growth rates reported by Keddy (1998) were measured from the time of the seedling germination and over a relatively short period (e.g. 6 weeks) compared to this study (i.e. 703 days). The RGR of plants grown in the system evaluated in this study were, as a consequence, lower than those values

published for wetland plants of similar niche, and supported the collateral results that indicated that the experimental plants existed in a low-available-nutrient wetland environment.

Investigations were completed on the concentration of nitrogen and phosphorus in *Baumea articulata* and *Schoenoplectus validus* plants originating from both natural and constructed wetlands (see sections 1.5 and 1.6) and the following text evaluate these results.

***Baumea articulata* at planting (polishing wetlands T4 - T6).** At the time of planting, the polishing wetland planting concentration of nitrogen in *Baumea articulata* samples was higher than reported for samples of *Baumea articulata* plants located in some natural wetlands (Adcock and Ganf, 1994), less than reported for others (Greenway, 1997), and lower than that reported for samples taken from constructed wetlands receiving dairy farm and secondarily treated municipal wastes(Tanner, 1996, Greenway, 1997). The *Baumea articulata* planting nitrogen values were similar though to that reported in plant samples taken from tertiary treated municipal wastewater wetlands (Adcock and Ganf, 1994). Consequently, the concentration of phosphorus in *Baumea articulata* plant samples was more representative of *Baumea articulata* plants sampled from natural wetlands (Adcock and Ganf, 1994), and less representative of plant samples taken from natural wetlands that received wastewater (Greenway, 1997), or treatment wetlands that receive tertiary and secondarily treated municipal wastewaters (Greenway, 1997, Adcock and Ganf, 1994), or dairy farm wastewater (Tanner, 1996).

Nevertheless, *Baumea articulata* plant sample nitrogen and phosphorus concentrations at the time of planting were within previously reported measured

ranges, and reflected plants originating from natural conditions (i.e. limited availability of nitrogen and phosphorus).

***Baumea articulata* at harvest (polishing wetland T4 – T6).** At harvest time, *Baumea articulata* polishing wetland plant sample concentrations of nitrogen were less than recorded at the time of planting (see table 2.8), but similar to nitrogen concentrations measured in plant samples taken from some natural wetlands, and constructed wetlands receiving tertiary municipal wastewater (Adcock and Ganf, 1994); and lower than those reported in *Baumea articulata* plants sampled from other natural wetlands (Greenway, 1997), and wetlands constructed to treat secondary municipal and dairy farm wastewaters (Tanner, 1996, Greenway, 1997). *Baumea articulata* plant sample phosphorus concentrations at wetland harvest were lower than at the time of planting (see table 2.8). When compared to published values, concentrations were similar to or lower than those reported for *Baumea articulata* plants sampled from natural wetlands (Greenway, 1997, Adcock and Ganf, 1994), and wastewater treatment wetlands receiving tertiary and secondarily treated municipal wastewaters (Greenway, 1997, Adcock and Ganf, 1994), or dairy farm wastewater (Tanner, 1996)

Although the concentrations of nitrogen and phosphorus declined from planting to harvest, *Baumea articulata* plant sample concentrations at the time of harvest were still within previously reported ranges, and reflected the condition of plants originating from natural conditions i.e. limited availability of nitrogen and phosphorus). Thus, nitrogen and phosphorus status of the *Baumea articulata* in this study was simply a reflection of the growing environment: the polishing wetland waters were limited in biologically available nitrogen and phosphorus, and possibly

other nutrients.

***Schoenoplectus validus* at planting (polishing wetlands T1 - T3).** At the time of planting, the polishing wetland concentration of nitrogen in *Schoenoplectus validus* plant samples was greater than that reported for *Schoenoplectus validus* plant samples originating from natural wetlands (Greenway, 1997) and less than reported for plants sampled from constructed wetlands receiving secondarily treated municipal wastewater or dairy farm wastewater (Tanner, 1996, Greenway, 1997, Greenway and Woolley, 1999). Concurrently, the concentration of phosphorus in *Schoenoplectus validus* plant samples was higher than in plants sampled from natural wetlands (Greenway, 1997), and more resembled that of plant samples taken from wastewater treatment wetlands receiving secondarily treated municipal wastewaters (Greenway, 1997, Greenway and Woolley, 1999), or dairy farm wastewater (Tanner, 1996).

Even so, *Schoenoplectus validus* plant sample nitrogen and phosphorus concentrations at the time of planting were within previously reported ranges, and reflected plants originating from more eutrophicated wastewater conditions - a result of earlier plant nursery growing conditions (nutrient conditioning).

***Schoenoplectus validus* at harvest (polishing wetlands T1 –T3).** At the time of wetland harvest, the plant sample concentrations of nitrogen and phosphorus were comparable to those reported for *Schoenoplectus validus* samples taken from natural wetlands (Greenway, 1997) , and lower than those reported for samples taken from constructed wetlands that received municipal secondary or dairy farm wastewaters (Greenway, 1997, Greenway and Woolley, 1999, Tanner, 1996). The concentrations of nitrogen and phosphorus declined from planting to harvest (see

table 2.8), *Schoenoplectus validus* plant sample concentrations at the time of harvest were still within previously measured ranges, and reflected plants growing in the nutrient limited conditions of the polishing wetlands (limited availability of nitrogen and phosphorus).

***Schoenoplectus validus* at planting (culture wetlands R1-R2).** The relative growth rate (RGR), relative surface area expansion rate (RSR), relative light penetration decrease rate (RLDR), and mean net photosynthetic (CO_2 fixation) rate of *Schoenoplectus validus* plants grown in the culture wetlands (R1 - R2) were greater than those of *Schoenoplectus validus* and *Baumea articulata* plants growing in polishing wetlands (T1 - T6). The more robust culture wetland plants were thought to have benefited by the warmer water temperatures and the greater availability of nutrients originating directly from the polycultured fish and crayfish, and other animal wastes upstream in the system.

At the time of planting of the culture wetland, the concentration of nitrogen in *Schoenoplectus validus* plant samples was less than that reported in plants sampled from constructed wetlands receiving secondarily treated municipal wastewater or dairy farm wastewater (Tanner, 1996, Greenway, 1997, Greenway and Woolley, 1999), but more similar to samples taken from natural wetlands (Greenway, 1997). At the same time, the concentration of phosphorus in *Schoenoplectus validus* plant samples was lower than that reported for plants sampled from natural wetlands (Greenway, 1997), wastewater treatment wetlands receiving secondarily treated municipal wastewaters (Greenway, 1997, Greenway and Woolley, 1999), and from dairy farm wastewater wetlands (Tanner, 1996).

Nonetheless, *Schoenoplectus validus* plant samples at the time of planting in

culture wetlands were within previously reported ranges, and reflected plants originating from natural conditions (i.e. limited availability of nitrogen and phosphorus).

***Schoenoplectus validus* at harvest (culture wetlands R1 – R2).** Culture wetland *Schoenoplectus validus* plant sample concentrations of nitrogen and phosphorus were greater at harvest than at the time of planting (see table 2.8). Furthermore, nitrogen in culture wetland *Schoenoplectus validus* plant samples was more concentrated than in plants sampled from natural wetlands (Greenway, 1997), but less concentrated than in plants sampled from constructed wetlands receiving dairy farm wastewater (Tanner, 1996). Plant samples were more representative of plants receiving secondarily treated municipal wastewater and (Greenway, 1997, Greenway and Woolley, 1999). Concurrently, phosphorus in culture wetland *Schoenoplectus validus* plant samples were more concentrated than in plants sampled from natural wetlands (Greenway, 1997), but were more representative of plants samples was constructed wetlands receiving dairy farm and wastewater secondarily treated municipal wastewater (Tanner, 1996Greenway, 1997 #40, Greenway and Woolley, 1999).

In summary, the concentrations of nitrogen and phosphorus in *Schoenoplectus validus* grown in culture wetlands increased from planting to harvest. Additionally, the concentrations of nitrogen and phosphorus in the *Schoenoplectus validus* harvested from the culture wetlands were greater than that recorded for either species of plant grown in polishing wetlands T1 - T6 as expected, considering that the culture wetlands received all the metabolic wastes generated over three fish and crayfish polyculture trials.

For both *Baumea articulata* and *Schoenoplectus validus* plants grown in polishing wetlands, the combination of slow growth and low nutrient concentrations was most akin to growth in natural wetlands, and was indicative of sub-optimal growing conditions. *Schoenoplectus validus* grown in culture wetlands had ample nitrogen and phosphorus tissue concentrations, and faired better than plants in the polishing wetlands. It is important to note that the N-P nutrient status of the plants harvested from the wetlands did not show the changes of plant tissue nutrient concentration over time. It is speculated the nutrient status of the plants would have been at its highest at the time of harvest, as harvesting took place in the summer season 25 days after the second polyculture trial concluded. Over the course of the experiment there was one long period (142 days) when fish culture (and other data collection) was suspended (and hence no feed or fish waste inputs to wetlands), and this would be expected to have been reflected in plant RGR as well as in tissue nutrient concentrations not quantified in the data set.

Algal harvest from polishing wetlands. Ephemeral algal blooms reoccurred in the polishing wetlands, and at the time of harvest the standing algal biomass collected was rich in nitrogen, with *c.* 19 times the concentration of nitrogen of the plants sampled from the wetlands at harvest (see digital appendix, folder - mass balance, files I and J). Algal phosphorus concentrations in were lower than that recorded for plants sampled from the wetlands at harvest (see digital appendix, folder - mass balance, files I and J). An algal sample was analyzed by the Centre for Environmental Management as containing filamentous green, and nitrogen fixing algae. Algal competition with symbio-nitrogen fixing *Azolla* spp. for phosphorus, micro-nutrients and light in the wetlands resulted in a semi-regular dysynchronous pattern of growth and decline of fern and algal species over the experiment. *Azolla*

spp. was not present in the wetlands at the time of harvest.

Algae were more effective at sequestering phosphorus in the *Schoenoplectus validus* wetlands than in the *Baumea articulata* wetlands. This was because *Schoenoplectus validus* did not grow as well as did the *Baumea articulata* under the environmental conditions and hence did not take up as much phosphorus; and algae were better able to co-exist with the *Schoenoplectus validus* for the nutrients and light.

2.7.7 Wetland *in-situ* electrochemical water quality

Within the culture wetland systems (e.g. fish tanks and corresponding culture wetlands) dissolved oxygen concentrations (measured at 10:00 and 4:00 pm daily) remained below saturation (Appendix F in Tchobanoglous, 1985 – see list of references) due to animal and bacterial respiration impacts. A maximum of ≤ 250 W h^{-1} of energy was required mechanically to aerate the wetlands and fish tanks to maintain the dissolved oxygen levels above 5.0 mg L^{-1} in the culture system at maximum fish density particularly at night when concentrations would be at their minima because of additional algal and plant root respiration. The *in-situ* concentrations of culture wetland DO, in this study, were between those reported in the literature for tilapia – red claw polyculture ponds (Rouse and Kahn, 1998)

The respiration in polishing wetlands was dominated by plant and algal needs and was not impacted by mechanical aeration and electrical heating. Therefore, oxygen concentrations (measured at 10:00 and 4:00 pm daily) were near

or slightly over saturation (Appendix F in Tchobanoglous, 1985 – see list of references) most likely because of photosynthetic production of oxygen by algae. Oxygen gas saturation levels between 102 - 103 % of normal can cause gas bubble disease in sensitive fish while 115-125 % of normal is lethal to most fish (Tchobanoglous, 1985). In contrast, although data were not collected during the night hours, it was expected that dissolved oxygen concentrations were low due to algal, bacterial, and root respiration (in the absence of mechanical aeration). The sharp drop in DO that occurred in the polishing wetlands (and to a lesser extent the culture wetlands) on October 4, 2001 (Figure 2.9) was not a function of temperature increase: the CR10X data logs showed that day to be overcast with a level of sunlight ranging between 47% and 58% of the levels measured on both the previous and next day. Thus the drop in DO was most likely due to a suppression of photosynthesis caused by reduced solar input.

Over the experiment, wetland pH values were within a *c.* 2 unit range. Initially, culture wetland pH was more variable between R1 and R2 wetlands and between sample dates. That variability tended to decrease over the trials 1 and 2, a result of increased canopy shading. The trend of decreasing variability cannot be extended to trial 3 as data between R1 and R2 were staggered in time because of an initial crop failure. The *in-situ* pH of culture wetlands was comparable to that as reported in tilapia – red claw polyculture ponds (Karplus et al., 2001)

In this experiment the null hypothesis was rejected in terms of *in-situ* electrochemical pH: maintaining a lower level in *Baumea articulata* wetlands. The ability of *Baumea articulata* wetlands to maintain a significantly lower pH than that of *Schoenoplectus validus* wetlands was considered to be related to the faster relative

light penetration decrease rate (RDLR) recorded for the *Baumea articulata* wetland plants. More shade produced by the *Baumea articulata* wetland canopies was thought to have slowed the rate of wetland algal photosynthesis when compared to that of algae found in the less-shaded *Schoenoplectus validus* wetlands. This would have reduced the extraction of CO_2 from the water column that otherwise would act to decrease pH during daylight hours in aquatic systems with dense algal populations - one apparent advantage of a fuller canopy.

The culture wetland canopies produced more than double the shade than did polishing wetland canopies by the end the second trial, and were thought to be efficiently converting ammonia to nitrate (lowering pH) (Tchobanoglous, 1985), and fish / bacterial respiration further acted to lower pH via CO_2 production. Surprisingly however, culture wetland pH was similar to *Schoenoplectus validus* polishing wetlands pH during the day. The reason for this similarity was thought to be related to the large water surface areas afforded by wetlands, and the aeration / agitation of the waters in the culture system efficiently absorbing and venting off the built up CO_2 in the water column, in addition to algal photosynthetic absorption of CO_2 from the water column during the day.

Concentrations of total dissolved solids in the wetlands increased over periods without rain or fresh water inputs, and then fell when those water additions occurred. The sharp increase in TDS seen in trial 2 (Figure 2.11) was caused by the release of approximately 300 L of residual barramundi salt-bathing water into each of the wetlands, which raised the TDS concentrations to the upper limit of what constitutes fresh water (Tchobanoglous, 1985). In trial 3, both R1 and R2 TDS concentrations ranged well within freshwater values (Tchobanoglous, 1985).

Decreases in TDS of wetland R1 over the final three months of the fish culture trial were in response to the regular freshwater irrigations estimated to be required to maintain oxygen levels above 5 mg L^{-1} in the face of increasing animal, bacterial, and plant respiration. With respect to aquaculture requirements, the water quality of TDS concentrations within the culture wetlands over the experiment remained within the ranges tolerated the primary and secondary aquaculture species, as well as other aquatic organisms living in the wetland.

Concentrations of total dissolved solids in polishing wetlands were greater than those of culture wetlands although the trends over time were the same for both (barring the salt water addition in trial 2). At the end of trial 2, polishing wetland TDS concentrations approached concentrations that would constitute moderately brackish water (Tchobanoglous, 1985) however the final batch flow event of trial 2 brought the TDS concentrations back to more appropriate freshwater values.

Water temperatures in all wetlands followed seasonal patterns. For culture wetlands temperatures were increased artificially by electrical heaters. Culture wetland water temperatures in trials 1 and 2 were close to those of polishing wetlands when heaters were turned off while the primary culture animal was not in the system (Figure 2.12). In trial 3, an electrical fire caused the termination of heat of R2 wetland-tank system in June, and in July, the heater in R1 was faulty until repaired in early August (Figure 2.16). The *in-situ* temperatures of culture wetlands were comparable to those reported in fish – crayfish polyculture ponds in the published literature (Rouse and Kahn, 1998, Karplus et al., 1995, Karplus et al., 2001).

2.7.8 Wetland nutrient water quality

In terms of aquaculture water quality, the concentrations of total nitrogen (TN) and total phosphorus (TP) in the Rockhampton city drinking water used to irrigate the system were low. However, when observing the ANZECC and ARMCANZ (2000) guidelines (designed to ensure low risk of adverse biological effects to natural aquatic ecosystems), the city water exceeded the maximum for TN and TP concentrations 20% of the time and therefore could not be considered a low risk if discharged into aquatic environments. Given the fact that the quality of the city water met potable water standards, and yet breached the ANZECC standards designed to protect natural ecosystems, the chances of the integrated polyculture system wastewater effluent meeting those standards all of the time was highly unlikely. It should be noted that due to the cost of municipal water, bore water and / or disinfected surface waters are likely candidates for use in aquaculture systems.

The rise in concentration of TN in culture wetland effluent was an impact of the feed inputs required to support the increasing culture fish density over trial 1, and peaked between the final water quality sample taken in trial 1 and the first sample taken in trial 2. Because trial 2 operated under lower fish culture densities, total nitrogen concentrations in culture wetlands declined under the lower nutrient load, maintaining a concentration close to, but above, the ANZECC trigger levels until the end of the trial (Figure 2.17). However, for most of the time, R1 and R2 culture wetland water could be classified as within the reported range of freshwaters with respect to total nitrogen (Boyd, 1979). It must be noted that the research from Boyd (1979) was undertaken in the USA, where nitrogen and phosphorus levels are likely higher than found in Australia. At its most concentrated, the total nitrogen levels

within culture wetland water were comparable to those found by Sansanayuth (1996) in the culture pond section of an integrated prawn culture and constructed wetlands system.

The oxidized nitrogen within the culture wetlands followed the same trends as the TN for it was the major constituent of TN in the culture wetland effluent. This was evidence that the wetlands efficiently bioremediated ammonia/ammonium produced in fish wastes and favored microbial decomposition of organic proteins in the system. However, denitrification (that was likely to be occurring in the wetland soil substrates) was not rapid enough to denitrify the amount of oxidized nitrogen that was concentrating in the wetlands. The denitrification process may have been retarded by the oxic conditions in the wetlands as well as the limited amount of detrital organic carbon present in the wetland soil substrates in the early stages of the constructed wetlands development. Oxidized nitrogen concentrations in the culture wetlands did reach levels than those reported for other integrated wetland aquaculture systems (Tilley et al., 2002) or reported for fish - crayfish polyculture systems (Rouse and Kahn, 1998), however they were well below those levels considered toxic to fish (ANZECC and ARMCANZ, 2000).

On one sample date, ammonium in the culture wetland was elevated. The spike in ammonium followed the TN and NOx spikes, although it was a small increase and only noticeable in comparison to the very low levels consistently found in the culture wetlands. Nonetheless, at all times, culture wetland ammonium concentrations were within those reported for integrated wetland aquaculture systems, and fish - crayfish polyculture systems at all times (Rouse and Kahn, 1998, Tilley et al., 2002, Karplus et al., 2001).

The concentration of TP in culture wetland effluent rose in response to the feed inputs required to support the increasing culture fish density over trial 1, and peaked, as for nitrogen concentration, between the final water quality sample taken in trial 1 and the first sample taken in trial 2. Because trial 2 involved lower fish culture densities, TP concentrations in culture wetland effluent declined, maintaining a concentration much closer and even below the ANZECC trigger level (Figure 2.17). Even so, the total phosphorus concentrations within culture wetland water were much higher than those reported by Sansanayuth (1996) for the culture pond section of an integrated prawn culture and constructed wetlands system. Such that the culture wetland waters could be classified to be in a hyper-eutrophic condition (Boyd, 1979).

The filterable reactive phosphorus (FRP) within the culture wetlands followed a trend that was similar to that of TP, however FRP constituted a minor component of TP in trial 1 (see figures 2.20 and 2.21). In trial 2, FRP constituted a greater proportion of the TP, posing a greater threat to the environment if discharged. It was envisaged that the phosphorus carrying capacity of biological components within the wetlands might be near carrying capacity by the time of initiation of trial 2. This, in addition to the ephemeral nature of the biological phosphorus reservoirs (e.g. dysynchronous algae / *Azolla* spp.), could explain why the reactive form of phosphorus was more available within the culture wetlands in trial 2 than in trial 1. Nevertheless, FRP concentrations of culture wetland did meet ANZECC / ARMCANZ (2000) guidelines over half of the time (the best result of all nutrients examined) hence wetlands were able to control the reactive form of phosphorus. Prior to this study no earlier reports exist in the literature of FRP measurements of the culture water sampled from integrated wetland aquaculture systems, and fish -

crayfish polyculture systems.

Concentrations of suspended solids in culture wetlands were much lower than those reported by Sansanayuth (1996) for the culture pond section of an integrated prawn culture and constructed wetlands system. Culture wetlands were very effective at keeping suspended solids to low concentrations.

With respect to goal 5, the concentration of total nitrogen (TN), oxidized nitrogen (NO_x), ammonium (NH_4^+), total phosphorus (TP), and filterable reactive phosphorus (FRP) leaving the system in wastewater did not consistently meet the water quality trigger levels suggested by the Australian and New Zealand Environment and Conservation Council (ANZECC), although suspended solids (SS) did meet the trigger level. Therefore, using terminal system effluent for land based agriculture and/or shunting it to an evaporation pond (as in this experiment) or terminal wetland (i.e. further treatment) would be necessary to prevent damage to the surrounding ecosystems. Another option is to decrease the initial pollution load via a reduction in fish density, and thereby decreasing profit potential.

The concentration of TN in polishing wetland effluent followed the same trend as it did in the culture wetlands although on a reduced scale (Figure 2.17). At all times, polishing wetland effluent water could be classified as within the range of freshwaters with respect to TN (Boyd, 1979). The total nitrogen concentration within effluents exiting the polishing wetland was lower than that found by Sansanayuth (1996) for the effluent of a treatment wetland integrated with prawn culture. The polishing wetlands removal of nitrogen entering from the culture wetlands effluent was nearly 85 %, that is 19 % greater than the removal of nitrogen reported for a constructed wetland used to treat catfish effluents (Schwartz and Boyd,

1995).

The oxidized nitrogen within the polishing wetlands effluent followed the same trend as in the culture wetlands although on a reduced scale (Figure 2.18). In comparison to culture wetland effluents, oxidized nitrogen constituted a small proportion of the TN in polishing wetlands effluent. The oxidized nitrogen entering the polishing wetlands in effluent was subsequently bound in organic biomass (e.g. algae and plant) and/or denitrified within the wetlands and/or vented to the atmosphere, leaving the polishing wetland effluent with a lower proportion of the TN existing as the bioactive NO_x configurations. Oxidized nitrogen concentrations in the polishing wetlands were within ranges of concentrations for wetland effluent water quality reported for integrated wetland and aquaculture systems (Barlow, 1998, Lin et al., 2003, Tilley et al., 2002, Costa-Pierce, 1998). In this experiment the null hypothesis (see section 2.1.3) was rejected in terms of oxidized nitrogen discharged in the wastewater effluent. The one occasion where *Schoenoplectus validus* wetland effluents had significantly lower concentrations of NO_x than did *Baumea articulata* wetland effluents was most likely due to the comparatively greater biomass of algae in *Schoenoplectus validus* wetlands at that time possibly in a stage of logarithmic growth (algal bloom).

The concentration of ammonium (NH_4^+) in wastewater entering the polishing wetlands was low, and that in the effluent leaving the polishing wetlands was lower still, suggesting that the NH_4^+ that entered the polishing wetlands was nitrified further, leaving residuals of ≤ 0.02 mg L^{-1} in the wastewater. Ammonium concentrations in the polishing wetlands were well below wetland effluent concentrations reported in integrated wetland and aquaculture systems (Tilley et al.,

2002, Costa-Pierce, 1998).

Polishing wetlands successfully sequestered or otherwise facilitated the removal of over 85 % of the TP entering in polyculture wastewater, maintaining relatively stable concentrations over the course of the experiment and ranging near or below the ANZECC / ARMCANZ (2000) trigger values. Total phosphorus concentrations in the polishing wetlands were within ranges reported for wetland effluent water quality for integrated wetland and aquaculture systems (Comeau et al., 2001, Sansanayuth et al., 1996). The polishing wetlands removal of TP entering from the culture wetlands effluent was comparable to that reported for a constructed wetland used to treat catfish culture effluents (Schwartz and Boyd, 1995).

The FRP within the polishing wetlands followed a trend that was similar to that of total phosphorus in this study. The effluent of polishing wetlands presented a smaller proportion of FRP in comparison to the culture wetland effluents. The FRP entering the polishing wetlands was quickly bound to organic and physical substrates (e.g. plant, algae, and soil), producing discharge effluent with a lower proportion of the TP existing as the bioactive configuration. With respect to the ANZECC guidelines, FRP faired best among the nutrients measured, meeting the trigger values in most instances. Thus, the polishing wetlands were able to effectively control the reactive form of phosphorus. Filterable reactive phosphorus concentrations in the polishing wetlands were well below wetland effluent concentrations reported in the literature for integrated wetland and aquaculture systems (Tilley et al., 2002, Lin et al., 2003).

Similarly, suspended solids concentrations in the polishing wetland effluents were well below wetland effluent concentrations reported for integrated wetland and

aquaculture systems (Tilley et al., 2002, Lin et al., 2003). Polishing wetlands were shown to be very effective at keeping suspended solids at low concentrations in this study.

2.7.9 Hydrology

High evapotranspiration rates and their concentrating-impact on water quality, compounded by the oxygen and feed requirements of the fish, were the driving factors of R1 and R2 culture wetland irrigation in trials 1 and 2. Evapotranspiration rates were 10 to 20 times that reported by Schwartz and Boyd (1995) for an integrated wetland aquaculture system. The disparity was most likely due to differences in climate and season, as that system was located in Alabama (USA) and operated over the winter season, in contrast to the previously described subtropical conditions of heat and drought experienced over the course of this study. Nearly 20% of the water required by the culture wetlands to support animal, plant, and biodiversity was met by rain capture (Figure 2.23). The 2 : 1 ratio of water usage (m^3) to fish produced (kg) cannot be compared with other systems because equivalent values have not been reported in the literature. Although it was not employed in this system, efficient rain capture off the roof of the shade house would have increased the efficiency of water use dramatically, and therefore should be a design feature of any future system. Trial 3 hydrology was similar to trials 1 and 2, although less rain fell and more water was used resulting in less than 10 % of the water required by the culture wetlands to support animal, plant, and biodiversity being met by rain capture.

Polyculture system discharges controlled the hydrology of the polishing wetlands in trials 1 and 2; however additions of fresh water were required due to high evapotranspiration rates over periods when animal culture was not taking place. Evapotranspiration rates for polishing wetlands were near those of culture wetlands in trials 1 and 2, however the culture wetlands were characterized by evapotranspiration rates 7 - 8 % higher because they were aerated.

2.7.10 Carbon - nitrogen – phosphorus (CNP) mass balance

At the conclusion of the experiment, large surpluses of carbon, and lesser surpluses of nitrogen and phosphorus were measured in the system., meaning that, there was more CNP measured bound within the wetland and combined in system outputs such as animals in wastewater than was measured entering the system. The surplus of carbon was due to the photosynthetic fixation of atmospheric and aqueous CO_2 by the plants and algae in the system, dually providing physical structures (e.g. plant stems and roots) for the wetland inhabitants to live on or in-between, and the carbon-based molecules that supported the wetland heterotrophic food web. A portion of surplus of TN and TP in the system could be attributed to system inputs that were not measured, such as atmospheric nitrogen gas fixed by algae and/or *Azolla* spp. and phosphorus inputs by frog and visiting biota wastes.

Overall, the system was very efficient in sequestering the TN and TP introduced in fish feed within the solid substrates (e.g. plant, fish, soil, algae), preventing the nutrients from traveling across the system boundary in discharge wastewater. When comparing the amount of TN and TP discharged into the

environment in relationship to fish feed inputs, the integrated system trialed in this experiment was literally hundreds of times more efficient than efficiencies calculated for reported freshwater catfish pond farming, or rainbow trout marine fish cage farming techniques (Holby et al., 1991, Hall et al., 1992, Schwartz and Boyd, 1994), or that described in standard aquaculture operations (Handy and Poxton, 1993, Black, 2001). Per kilogram of fish produced, the amount of TN and TP lost to the environment in wastewater also was much lower in this experiment than reported elsewhere (Schwartz and Boyd, 1994, Costa-Pierce, 1996).

The remainder of this section discusses the system mass balance by individual substrate in more detail.

Lates calcarifer. In this experiment fish were the greatest sink for CNP export from the system. Recovery of food input TC by the barramundi of this study was more than twice that reported for cage cultured marine rainbow trout (Hall et al., 1990). Recovery of food input TN by the barramundi was equal to TN recoveries reported for silver perch and tilapia grown in freshwater aquaponic systems (Zweig, 1986, Holland); and approximated that reported for another tilapia freshwater aquaponic system (Quielleré et al., 1995), while being 10 % higher in comparison to a marine rainbow trout cage system (Hall et al., 1992). The amount of TP sequestered from fish feed inputs by the barramundi was within 5 % of that reported for aquaponic silver perch (*Bidyanus bidyanus*) (Holland, 2002) but twice as much as that reported for cage-cultured marine rainbow trout (Hall et al., 1990). Thus, the efficiency of barramundi to sequester nitrogen and phosphorus from food was comparable to that of other freshwater cultured species, but more efficient than marine cage-cultured rainbow trout. The differences between marine and freshwater examples could be

the result of metabolic differences between species undoubtedly influenced by geography and culture methods and conditions.

Cherax quadricarinatus. Red claw represented the second largest sink for CNP export from the system. There are no other reports of red claw (or other crayfish) mass balance in the literature. In this experiment, red claw were able to sequester *c.* 17 times more TN and 14 times more TP than was released in the systems wastewater; providing an ecological service that not only helped to maintain the water quality of the wetlands via scavenging plant and animal matter litter, but produced a marketable source of high protein at production costs restricted to harvest and post-harvest procedures only.

Velesunio ambiguus. With respect to CNP input, mussels represented a very small fraction in comparison to other substrates (e.g. fish, plants, soil), because they did not persist in the wetlands past trial 2.

Wetland plants. Wetland plants played a key role in the sequestration of input nitrogen and phosphorous, even though estimates of wetland plant nitrogen sequestration in comparison to feed inputs may be artificially high as *in-situ* wetland nitrogen fixation by *Azolla* spp. and algae may have released nitrogen into the water column via decomposition upon death or animal metabolism (e.g. crayfish and snails). The nutrient-enriched status of culture wetland plants in comparison to polishing wetland plants was undoubtedly due to the nutrient rich environment produced by the animal culture wastes.

Although *Schoenoplectus validus* plants harvested from the polishing wetlands had statistically higher concentrations of nitrogen, and non-statistically

significantly higher concentrations of phosphorus than did *Baumea articulata* plants, they were characteristic of *Schoenoplectus validus* plants taken from nutrient limited environments, and they grew at a slower rate than did *Baumea articulata*. Additionally, harvested *Baumea articulata* were more characteristic of plants taken from wastewater constructed wetlands and natural wetlands. This suggested that *Baumea articulata* had a greater capacity to flourish in limited nutrient conditions found in free-surface polishing wetlands designed to manage integrated polyculture wastewater under similar conditions.

Wetland algae. Wetland algal biomass held more than 10% of the nitrogen, 5% of the carbon, but negligible amounts of the phosphorus recovered from the system. Algal growth was successful to such a degree from a system-wide perspective because of its dominance of the *Schoenoplectus validus* polishing wetlands CNP mass balance, and to a lesser degree, within the *Baumea articulata* polishing wetlands CNP mass balance.

The fact that the *Schoenoplectus validus* was less effective at competing with the algae (and other competitors) for the limited nutrients available in the polishing wetlands than *Baumea articulata* was supported by the lower RGR measured in *Schoenoplectus validus* and by its harvest nutrient composition being characteristic of plants existing in nutrient limited environments. It is predicted that the algal dominance in the polishing wetlands water column would be eliminated by the plant canopies of *Schoenoplectus validus* shading out the algae if the experiment were prolonged by *c.* 2 years.

Wetland substrates. The amount of CNP accumulated in the substrates related to feed inputs was lower in this system when compared to what was found in the

substrates directly underneath the cages of marine cultured rainbow trout (Hall et al., 1990). The amount of phosphorus sequestered in the systems combined wetland substrates (0.7 g m^2 yr^{-1}) was moderately higher than that suggested by Richardson (1993) to be the permanent storage of phosphorus in wetlands (generally below 0.5 g m^2 yr^{-1}) implicating that at least some of the TP in the substrate of the wetlands was most likely as a biomass component, such as live or non-decomposed algae and plant components of the soil, or non-mineralized benthic fish sludge. The nutrient-enriched status of culture wetland substrate when compared to polishing wetlands was undoubtedly due to the nutrient rich environment produced by the animal culture wastes.

Wastewater. Polishing wetland wastewater held the least significant amounts of the nitrogen and phosphorus recovered from the system, discharging far less than 10 % of the total nitrogen, phosphorus and carbon that was measured entering the system as fish feed, hence meeting performance goal 4.

In summary, the wetlands in the system captured or otherwise remediated input nitrogen and phosphorus that would have been left in the wastewater. Although the wastewater discharged from the polishing in wetlands was captured in an evaporation basin, it represented environmental discharge for the purpose of this experiment. As discussed previously in this section, in comparison to other reports of aquaculture mass balance, the current system was still hundreds of times more efficient in keeping influent nitrogen and phosphorus from entering the environment as direct metabolic wastes (as occurs in cage culture) or in wastewater discharge (as characterizes land based systems).

author	animal cultured	system type	days	stocking weight (g)	harvest weight (g)	growth rate (SGR)	feeding rate % bw d^{-1}	FCR
Brummet (1994)	*Oreochromis niloticus*	plant invaded pond	100	54	185 - 207	-	2.5 - 7.5	1.38 - 8.7 : 1
	Cherax quadricarinatus	plant invaded pond	170	2 -5	54 - 56 g	0.034-0.037	2.5 - 7.5	-
Schwartz and Boyd (1995)	*Ictalurus punctatus*	culture pond + wetland recirculation	-	-	-	-	-	-
Costa-Pierce (1998) [1]	*Oreochromis mossambicus* x *O. urolepis hornorum*	culture pond + wetland recirculation	202	21	362 - 404	1.44 - 1.66	1.0	0.9 - 2.3 : 1
	Cyprinus carpio	culture pond + wetland recirculation	202	456	611 - 703	0.11 - .021	1.0	0.9 - 2.3 : 1
Comeau (2001)	freshwater trout	treatment wetland	540	-	-	-	-	-
Lin (2003)	*Litopenaeus vannamei*	culture pond + wetland recirculation	80	-	-	-	-	-
Tilley (2002)	*Litopenaeus vannamei*	culture pond + wetland recirculation	365	-	-	-	-	-
Sansanayuth (1996)	*Macrobrachium rosenbergii*	treatment wetland	30	-	-	-	-	-
Gautier (2001)	*Litopenaeus vannamei*	natural wetland	-	-	-	-	-	-
Roe (2004)	*Lates calcarifer*	Culture tank + Wetland recirculation + polishing wetland *	123-147	10 - 13	274 - 520	2.42 – 2.79	2.3 – 2.6	0.8 - 1.0 : 1
Trials 1 - 3	*Cherax quadricarinatus*		146-211	3 - 18	13 - 80	0.92 – 0.97	nil	not fed

(Table 2.12 continued below)

Table 2.12. Integrated wetland – aquaculture systems reported in the literature, delineated by author, animal cultured, system type, days of activity, animal stocking weight (g), animal harvest weight (g), growth rate (SGR), feeding rate % bw d^{-1}, and food conversion ratio. Stocking and harvest weights (g) are ranges for individual animals. The symbol (-) represents no data reported. [1] Gambusia affinis and Procambarus clarkii were also include in the system, although performance values were not reported.

* polishing wetlands online for trials 1 and 2 only.

(Table 2.12 continued)

author	max culture density (g m^2)	temp °C	survival %	*in-situ* water quality (mg L^{-1})	effluent water quality (mg L^{-1})	wetland pollutant removal (%)	ET % of water inputs	ratio of wetland to pond area (m^2)	diversity
Brummet (1994)	163 - 175	-	82 - 85	-	-	-	-	-	alligators, egrets frogs, herons, raccoons and snakes
	50 - 59	-	36 - 45	-	-	-	-	-	-
Schwartz and Boyd (1995)	-	-		-	-	TKN 45-61 TAN 1-81 NO_2 43 -98 NO_3 51-75 TP 49-84 SS 57-100 TSS 75 - 87 VSS 68 -91	2.8 - 5.8	0.7 - 2.7 : 1	-
Costa-Pierce (1998)[1]	8	20	48 - 64	-	NH_3 0.4 NO_3 nil (wetland)	NH_3 90 (pond) NO_3 90 (pond) NH_3 7 (wetland) NO_3 7 (wetland)	-	-	threatened birds
	8	20	88 - 100	-	-	-	-	-	-
Comeau (2001)	-	-		-	TP < 0.1 SS < 4.0	-	-	-	-
Lin (2003)	-	-	-	-	TAN 0.09 NO_3 012 NO_2 0.004 FRP 7.99 SS 10	-	-	-	-
Tilley (2002)	-	-	-	NH_3 < 0.4 NO_3 < 2.0	NO_3 < 2.0 NH_3 < 3.0 FRP < 0.2 TP < 0.7 SS < 150	-	-	-	-
Sansanayuth (1996)	-	-	-	TN 4.4 - 4.8 TP 0.15 - 0.17 SS 44 - 55 Temp °C 23-28	TN 1.7 - 2.4, TP 0.04 - 0.11 SS 5 - 11 Temp °C 24-27	-	-	-	-
Gautier (2001)	-	-	-	-	-	SS 10 FRP 0.02-0.8 TAN 0.01- 0.5 NO_3 0.4-1.2 Temp °C 27-29 pH 7.0-7.5 DO nil - 1.0	-	-	wetland birds
Roe (2004)	1150 – 2300 *	27	84 - 99	DO 3.8 - 9.0 pH 6.8 – 9.5 TDS 147 – 1684 Temp °C 9 – 33 TN 0.012 – 3.89 NO_3 0.006 – 3.75 NH_4 0.006 – 0.65 TP .0.14 – 1.37 FRP 0.001– 0.37 SS 0.3 – 1.4	Polishing wetlands DO 2.3 – 13.2 pH 6.8 – 9.8 TDS 137 - 2408 Temp °C 12 – 33 TN 0.012 – 0.940 NO_3 nil – 1.06 NH_4 nil – 0.17 TP 0.006 – 0.252 FRP nil- 0.055 SS 1 - 3	Polishing wetlands TN – 84.2 TP – 86.4	58 – 66 **	10.8 - 21.5 : 1 ***	Birds, frogs, geckos, spiders, ants, honey bees, benthic invert Nil cane toads
	82 -163 *	24 - 27	68 - 75						

Table 2.12 (continued). Integrated wetland – aquaculture systems reported in the literature, delineated by author, maximum animal culture density (g m^2), water temperature (°C), survival (%), in-situ water quality(mg L^{-1}), effluent water quality (mg L^{-1}), wetland pollutant removal (%), evapotranspiration (% of water inputs), ratio of pond area to wetland area (m^2), and diversity. ET = evapotranspiration

[1] *Gambusia affinis* and *Procambarus clarkii* were also include in the system, although performance values were not reported.
* Greater value includes culture wetland surface area; lower value includes polishing wetland surface area as well.
** Greater value includes culture wetland ET; lower value includes polishing wetland ET.
*** Lower value includes culture wetland surface area; higher value includes polishing wetland surface area as well.

Author	animal cultured	system type	days	stocking weight (g)	harvest weight (g)	growth rate g d^{-1}
Karplus (2001)	*Oreochromis niloticus*	tank + biofilter	133	20 - 21	271 - 301	1.9 - 2.1
	Cherax quadricarinatus (males)	tank + biofilter	98	7	31-36	.018 - .021
Rouse, David B.	*Oreochromis mossambicus x O. urolepis hornorum*	pond	135	19	403-444	-
	Cherax quadricarinatus	pond	135	7	48-76	0.3-0.5
Karplus (1995)	*Cherax quadricarinatus*	pond	92	3	33	0.31 - .035
	Oreochromis niloticus x O. aureus	pond	92	23	172	1.6
	Cyprinus carpio	pond	92	131	561	4.7
	Hypophthalmichthys molitrix	pond	92	364	1037	7.3
Roe (2004) Trials 1 - 3	*Lates calcarifer*	tank and wetland	123 - 147	10 - 13	274 - 520	2.42 – 2.79 SGR
	Cherax quadricarinatus	wetland	146 - 211	3 - 18	13 - 80	0.05 - 0.40

(Table 2.13 continued below)

Table 2.13: Fish and crayfish polyculture systems reported in the literature, delineated by author, animal, system type, days of activity, animal stocking weight (g), animal harvest weight (g), growth rate.

(Table 2.13 continued)

author	animal cultured	feeding rate % bw d^{-1}	FCR	max culture density ($g\ m^2$)	temp °C	survival %	*in-situ* water quality (mg L^{-1}) unless otherwise stated
Karplus (2001)	*Oreochromis niloticus*	Zoah (1986)	1.67 - 1.71	5292 - 5420	20-30	92 - 95	DO 81 % sat, Ph 7.7 - 8.2 NH_4 0.06
	Cherax quadricarinatus (males)	4	-	187 - 403	20-30	57-60	DO 81 % sat, Ph 7.7 - 8.2 NH_4 0.06
Rouse (1998)	*Oreochromis mossambicus* x *O. urolepis hornorum*	3-5	2.5-8.1	194-326	23-33	84-90	NH_3 0.02 - 0.06 NO_3 < 0.05
	Cherax quadricarinatus	3-5 + 250 kg ha^{-1} every 14 d	2.5-8.0	18-35	23-34	19-24	DO 2.4 - 12.5
Karplus (1995)	*Cherax quadricarinatus*	-	-	-	21 - 31	22.3 – 27	-
	Oreochromis niloticus x *O. aureus*	-	-	-	21 - 31	90	-
	Cyprinus carpio	-	-	-	21 - 31	94	-
	Hypophthalmichthys molitrix	-	-	-	21 - 31	80	-
Roe (2004)	*Lates calcarifer*	2.3 – 2.6	0.8 - 1.0 : 1	2300–1150 *	27	84 - 99	DO 3.8 - 9.0 pH 6.8 – 9.5 TDS 147 – 1684 Temp °C 9 – 33 TN 0.012 – 3.89 NO_x 0.006 – 3.75 NH_4 0.006 – 0.65 TP .0.14 – 1.37 FRP 0.001– 0.37 SS 0.3 – 1.4
Trials 1 - 3	*Cherax quadricarinatus*	nil	not fed	163-82 *	24 - 27	68 - 75	

Table 2.13: Fish and crayfish polyculture systems reported in the literature, delineated by author, animal cultured, feeding rate (% bw d^{-1}), food conversion ratio (FCR), maximum culti density ($g\ m^2$), temperature (°C), survival %, and *in-situ* water quality.

* Greater value includes culture wetland surface area; lower value includes polishing wetland surface area as well.

2.8 Conclusion

This first known attempt to integrate barramundi culture, red claw culture, and constructed wetlands was successful as three healthy crops of commercially acceptable animals were produced. The highest production rate trial met commercial fiscal requirements with a water usage (m^3) to fish production (kg) ratio of 1.2 : 1. Barramundi grew efficiently in comparison to barramundi growth reported in the literature. Red claw grew less efficiently than those mono-cultured and fed directly, but their growth was efficient when compared to direct-fed red claw grown in other polyculture (mixed with fish) and/or wetland integrated systems. Further investigation of *Azolla* spp. as a feed source for red claw in polyculture wetlands is required, because it appeared that the floating aquatic fern was responsible for at least meeting partially red claw nutritional needs in this study and in this way contributed to the net surplus of system nitrogen via fixation of nitrogen gas. Freshwater mussels functioned in the system, however stocking of mussels in an established constructed wetland system (in contrast to a recently constructed wetland), and maintaining densities lower than trialed in this study (with respect to fish density and wetland surface area) may protect the mussels from starvation. However, the impact of a healthy (possibly procreating) mussel population upon the function of an integrated wetland polyculture system (e.g. fish and crayfish growth, water quality, etc) is unknown. Parasitic infections in this system were neither uncharacteristic of fish culture nor (importantly) serious in this study; however the issue of parasites residing within the wetlands, and their potential impact, is one that requires further investigation.

Employing wetlands as in-line and discharge water quality control ecosystems negating the need for all other water quality control devices (barring with the exception of

oxygenation) is exclusive to this study. Once constructed, the wetlands did not require further labor, electrical, or other maintenance inputs, making the method well suited in instances where capital investment, technology, electricity, materials, and/or skilled labor are limited. Water quality within the system was of high standards in terms of aquaculture requirements; however, if observing ANZECC guidelines, the terminal wastewater from the system required the evaporation basin as its final destination to consistently avoid threatening adjacent aquatic ecosystems with nitrogen and phosphorus inputs. It should be noted that wetlands can also be used as evaporation basins if designed and managed carefully. Integrated systems that incorporate non-environmental, or ecologically discharge options are better positioned to ensure licensing from, and compliance with, state and Federal regulation agencies.

In this wetland system, the plant species *Baumea articulata* performed better than *Schoenoplectus validus*. *Baumea articulata* grew faster and produced more biomass from culture wastes and green house gas absorption than *Schoenoplectus validus*, resulting in enhanced water quality and denser frog populations of higher density by comparison. Additionally, the nitrogen and phosphorus status of both plant species in polishing wetlands at the time of harvest resembled plants originating from natural wetland ecosystems. Hence, the accumulative results indicated that *Baumea articulata* plants were better able to tolerate the nutrient limited and biologically competitive polishing wetland environments. It was therefore recommended that *Baumea articulata* be used instead of *Schoenoplectus validus* in similar polishing wetland applications particularly when nutrient-limited conditions are expected. However, this experiment lasted only 703 days, and advantages one species might have over another in polishing wetlands after both species reach maximum biomass are unknown. It should also be noted that harvesting of the plant biomass after maximum biomass is reached would most likely be required to

remove accumulated organic nitrogen and phosphorus from the system, and allow for further sequestration of nitrogen and phosphorus from wastewater in new plant growth.

It is difficult to put a monetary or regulatory value on biodiversity due to its complexity and associated problems in costing and valuing, nevertheless its utmost importance to human kind is becoming generally accepted by those with access to mass communication. Some of the biodiversity values of the constructed wetlands in this system were measured, showing that they do attract biodiversity, supporting microbial to vertebrate species via the utilization of wastewater nutrients, sunlight, and atmospheric carbon dioxide; and it is likely that their ecology impacted the biodiversity outside the experimental community boundaries. Employing constructed wetlands in urbanized aquaculture is highly relevant as the biodiversity in those areas are more apt to have diverged from natural habitats. Additional investigation as to the economics of wetland biodiversity and how constructed wetlands impact existing rural and urban biodiversity are required.

A nitrogen, phosphorus, and to a lesser degree, carbon, mass balance was completed on several aspects of the integrated polyculture system. The system was highly efficient at assimilating input CNP and discharging negligible amounts of nitrogen and phosphorus in its wastewater in comparison to fish feed inputs. The mass balance was an effective way to determine the ultimate substrate partitioning of the CNP within the system, showing the efficiency at which each substrate (e.g. fish, crayfish, soil, plants, and wastewater) was able to sequester carbon, nitrogen, and phosphorus, and in what chemical configuration the nutrients existed (e.g. nitrates or ammonia in water). The efficiency at which the system chemical mass balance operated affected all parts of the system, most notably water quality and animal health. In this experiment, the CNP mass balance also

was employed successfully to establish differences between polishing wetland treatment plant species.

Certain gaps in knowledge of integrated - wetland aquaculture (outlined in section 1.8,, 1.11, 1.12) were filled. This study provided a detailed description of a wetland – aquaculture integrated system, giving definitive recommendations on both animal and plant aspects of the systems. It is now known that red claw can be cultured as true custodial niche dwellers in wetlands, with their growth, survival and reproduction supported exclusively by fish wastes that were (as a byproduct of water quality enhancement) converted to natural food sources in the wetlands. The physical shelter the wetland plants provided also was important in supporting the red claw.

Additionally, the data from this experiment were used (in combination with previously published data) to construct both predictive dynamic and static integrated system models equipped with a layperson user interface (discussed in detail within Chapter V - Dynamic system modeling).

The methods and knowledge base for the integration of wetlands with polyculture remain in their infancy although this study represents the most comprehensive systems approach to investigation of integrated systems among currently published examples of constructed wetland integration with aquaculture, and fish and crayfish polyculture. The results of this research experiment were useful and encouraging, and will hopefully lead to future applied research in ecologically engineered integrated primary production systems.