Vol. 186: 271-281, 1999 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published September 17

Nitrogen budgets for the areolated grouper *Epinephelus areolatus* cultured under laboratory conditions and in open-sea cages

K. M. Y. Leung[*], J. C. W. Chu, R. S. S. Wu[**]

Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

ABSTRACT: The nitrogen budget of grouper *Epinephelus areolatus* (Forskål) in a culture system can be represented by the mass balance equation: consumption (C) = feed input (I) − feed wastage (W) = production (P) + mortality (M) + excretion (E) + faecal production (F). A nitrogen budget was constructed for individual groupers cultured for 1 mo under laboratory conditions; and an annual nitrogen budget was also constructed for a cohort of *E. areolatus* cultured in an open-sea-cage farm and fed with trash fish. Consumption was determined independently both by feeding experiments as well as by summation of P, M, E and F, to provide an estimate of the accuracy of the budget equation. In the laboratory budget, 27.5% of N consumed was channelled to growth, while 64.4% was excreted in the form of ammonia and 8.1% lost in faeces. The N-assimilation efficiency was 91.9%, while the net N-retention efficiency was 29.9%. For the annual budget constructed for the open-sea-cage farm, only 8.6% of total N input into the farm was harvested in the form of fish production, while loss to mortality was 3.7%. Ammonia excretion was the most important N loss (46.0%), followed by feed wastage (37.7%) and faecal production (4.0%). 66% of total N input could be accounted for in the laboratory N budget for individual groupers, but only 48% of N input into the culture system could be accounted for in the annual nitrogen budget constructed for open-sea-cage farming. It was estimated that 87.7% of the total N input to the farm was lost to the environment (equivalent to 321 kg N t^{-1} of fish production). Such a value is almost 3 times as high as N loss from temperate salmonid farms.

KEY WORDS: Nitrogen budget · Assimilation efficiency · Grouper

INTRODUCTION

Marine fish culture in the coastal waters of many countries has grown dramatically in recent years, and further growth is expected in the coming decade (FAO 1995, New & Csavas 1995). In open-sea-cage culture, high organic and nutrient loadings generated from feed wastage, excretion and faecal production are directly discharged into the environment (Duff 1987, Hammo 1987, Waldichuk 1987, Wu 1988, 1995, Wildish et al. 1990, Soley et al. 1994, Wu et al. 1994). Consequently, there has been a growing concern over the impact of marine fish farming activities on water and sediment quality of the receiving marine environment (Ackefors 1986, Gowen & Bradbury 1987, Hakanson et al. 1988, Rosenthal et al. 1988, Ackefors & Enell 1990, Wu et al. 1994, Wu 1995). Indeed, environmental concern has already led to a moratorium on new developments and tighter controls in Denmark, Norway, New Zealand, Canada and Hong Kong (Duff 1987, BC Ministry of Environment 1990).

Nitrogen is often the limiting nutrient for primary production in coastal ecosystems (Dugdale 1967, Gunderson 1981, Cockcroft & McLachlan 1993). Ammonia and urea excreted by fish can be readily taken up by phytoplankton, and hence may promote algal blooms. It should also be noted that fish excreta and wasted food have a N:P ratio close to 7:1 (the Redfield ratio) (Aure & Stigebrandt

---

[*] Present address: Institute of Biomedical and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, Scotland, UK

[**] Addressee for correspondence.
E-mail: bhrswu@cityu.edu.hk

1990), and hence would provide well-balanced nutrients for phytoplankton growth. A number of studies have shown that excessive nitrogen caused by marine fish farming activities may lead to eutrophication and hence mortality of fish and benthos (Jones et al. 1982, Skogheim & Bremnes 1984, Phillips et al. 1985, Skjoldal & Dundas 1991). Eloranta & Palomaeki (1986) demonstrated an increase in phytoplankton biomass, chlorophyll *a*, and primary production in response to nutrient loading from fish farms. In Hong Kong, higher levels of nutrients (ammonia, nitrite, nitrate, inorganic phosphate) as well as phytoplankton numbers have been reported in fish culture zones (Wu 1988). In a review, Handy & Poxton (1993) estimated that 52 to 95% of N input into a fish culture system will ultimately be lost into the environment. Likewise, a study of the flux and mass balance of nitrogen in a rainbow trout cage farm where dry feed was used showed that 76% of the nitrogen feed input into the system was lost to the environment, and the total environmental loss of nitrogen was estimated at between 95 and 102 kg N t^{-1} production (Hall et al. 1992). Such a high N loading generated from the marine fish culture industry has obviously become a prime environmental concern.

Understanding the nitrogen budget of fish and fish farms is useful in mariculture management, since information on the loading and forms of nitrogen from various sources could enable appropriate measures to be devised for the sustainable development of the industry. For example, the quantity and forms of nitrogen loading generated from fish farms are essential in estimating the carrying capacity of culture waters and assessing the environmental impact; species with a low nitrogen loading may be selected for culture in areas with low flushing rates and where background levels of nitrogen are high; feed types may also be varied to reduce wastage if this is an important contributing factor to nitrogen loading. The great majority of existing data on N excretion and N budgets of fish, however, were derived from temperate species (mostly salmonids) (Handy & Poxton 1993). Nitrogen utilisation and loading of non-salmonid marine species are poorly known. Furthermore, most studies of nitrogen budgets of fish have been conducted in land-based culture facilities or laboratory systems, in which artificial feed is used (for a review, see Gowen & Bradbury 1987, Handy & Poxton 1993). So far, only a single study has been carried out in Sweden to estimate nitrogen loading for rainbow trout cultured in open-sea cages (Hall et al. 1992). Despite over 84% of global aquaculture production being derived from Asia, where the growth of marine fish farming is also the most rapid in the world (FAO 1995), the loading, sources, forms and fates of nitrogen generated from tropical and sub-tropical fish farms are virtually unknown.

It should be noted that there are fundamental differences in culture species, feed types and water temperatures between fish culture in temperate and tropical/sub-tropical countries (Wu et al. 1994). First, nitrogen requirements, metabolism and excretion of tropical and sub-tropical culture species (e.g. grouper, seabream, snapper) may be very different from their temperate counterparts (mostly salmonids). Second, unlike temperate countries (e.g. Scotland, Norway and Canada) where pellet feed is used, trash fish are fed in tropical/sub-tropical fish farms (e.g. Hong Kong, Thailand, Japan and Singapore). Feed wastage and nutrient loading are expected to be much higher when trash fish are used compared with fish farms where pellet feed is used (Warrer-Hansen 1982, Chu et al. 1995). Third, a higher water temperature regime in the tropics and sub-tropics may lead to a higher metabolic rate of fish and hence higher nitrogen utilisation. The paucity of data on tropical/sub-tropical species and culture systems, however, does not permit an accurate estimation of nitrogen loadings in open-sea-cage farms, which is common practise in this region.

The objectives of the present study are: (1) to construct a laboratory N budget for the areolated grouper *Epinephelus areolatus* (Forskål), a common culture species in tropical and sub-tropical regions, under controlled laboratory conditions; and (2) to construct an annual N budget for the same species cultured in open-sea cages. Despite the fact that large errors are expected to be incurred in nutrient budget studies, all reported nutrient budgets do not involve the independent measurement of individual budget items, and the errors involved hence cannot be estimated. In the present study, each budget item was measured independently, to estimate the balance of the budget, and also to shed light on the possible errors incurred in other nitrogen budgets.

MATERIALS AND METHODS

Terminology. The nitrogen budget of an individual fish or a fish farm can be represented by the following mass balance equation, in which each budget item is expressed in term of the mass of nitrogen.

$$C = P + M + E + F \qquad (1)$$

where C = N consumption; P = N retained for growth; M = N loss through mortality (in case of individual fish, M = 0); E = N loss through excretion; and F = N loss through faecal production. Assimilation efficiency (K_1) and nitrogen retention efficiency (K_2) for individual fish can be determined using the following equations:

$$K_1 = (C - F/C) \times 100\% \qquad (2)$$

$$K_2 = [P/(C - F)] \times 100\% \quad (3)$$

The nitrogen budget of a fish farm can be represented by the equation:

$$C = I - W \quad (4)$$

where I = total N input into the culture system; W = N loss through feed wastage; and C = N consumption by the culture stock.

Theoretically, the nitrogen budget equation (Eq. 1) should be balanced, and consumption C can be estimated from the summation of P, M, E and F. Similarly, any item in the equation which is difficult to measure can be found by the difference, provided that values of all other budget items are known. However, it is desirable to determine all budget items independently, since this will provide an independent check on the balance of the budget equation. The balance of the equation can be checked by comparing the consumption value derived from the summation method (C_S), with actual consumption values obtained directly from feeding experiments (C_F) using the following equation:

$$\% \text{ balance} = (C_S/C_F) \times 100\% \quad (5)$$

Laboratory N budget for individual *Epinephelus areolatus*. Groupers *Epinephelus areolatus* obtained from a local fish farm were acclimated in continuous flow seawater (temperature: 25 ± 1°C; salinity: 30‰) for 14 d. During the acclimation period, fish were fed with minced trash fish (*Sardinella* spp. and *Stolephorus* spp.; water content: 69.2 ± 1.5%, total N: 10.5 ± 3.4%) to satiation once daily. After acclimation, 8 fish (mean weight: 92.8 ± 9.6 g) were selected for the experiment. Each fish was reared individually in a 30 l tank with continuous flow for 1 mo under the same conditions and hand fed with trash fish to satiation once daily. Great care was exercised to ensure that no feed wastage was left on the bottom of each tank, and the amount of feed supplied to each fish was recorded. Seawater flow was stopped during feeding, and water was siphoned from each tank before and 10 to 15 min after feeding (to avoid biased estimate resulting from regurgitation, water samples were analysed for nitrite, nitrate, ammonia and total organic nitrogen, following the methods given in Strickland & Parsons (1972). Nitrogen loss in feed wastage (W) was calculated based on the increase in total N in each tank after feeding, and expressed as a percentage of N input. Consumption (C_F) was then calculated by the difference between input (I) and feed wastage (W).

Water content of trash fish was determined by difference in wet weight and dry weight, after drying the fish sample at 80°C for 48 h until constant weight was obtained. N content in the dried trash fish was then determined using a CHN analyser (CHN-900, Model 600-800-300, LECO® Corporation), and the amount of N in the feed was calculated and expressed as mg N g^{-1} dry wt. Weight gain for each individual fish was determined after 1 mo. Water and N content of the grouper carcasses were determined at the end of experiment, using the same methods described for trash fish. Production (P) was then estimated by the net gain of N in fish tissue.

Results of an earlier experiment showed that ammonia-N is the predominant excretory product in *Epinephelus areolatus* while TON and urea excretion are not detectable (Leung et al. 1999). Ammonical-N excretion rate (E) (mg N kg^{-1} d^{-1}) at 25°C was determined using the method and system described in Leung et al. (1999), and total N loss to excretion was calculated for the whole period.

Faecal matter in each metabolism chamber was also collected daily, and dried, weighed and analysed for N content. Faecal N production rate (F) was calculated and expressed as mg N kg $fish^{-1}$ d^{-1}. E and F for individual fish over the 1 mo experimental period were estimated by integrating daily excretion rates and faecal production rates over time.

Field N budget for *Epinephelus areolatus* cultured in open-sea cages. A 1 yr field study was carried out at the fish farm of the Kat O Fisheries Research Station, Hong Kong, to estimate the various budget items for groupers cultured in an open-sea-cage-farm. Four hundred *Epinephelus areolatus* fry (10 to 14 cm, fork length) were divided in equal number and put into 4 replicate sea cages (1.5 m × 0.75 m × 1.5 m: length × width × depth). Such a stocking density is typical in Hong Kong. Fish in each replicate cage were fed to satiation daily with trash fish, and the amount of trash fish supplied to each cage recorded. Nitrogen content of trash fish was determined for every month as described before, and N in feed input (I) was estimated from the amount of feed supplied to each cage and the nitrogen content of trash fish. Feed wastage was estimated by collecting unconsumed feed using a waste trap designed by Chu et al. (1995). This design involved suspending a polyethylene bag at the bottom of the cage. The bag tapered into a conical end at which a collecting bottle was secured. Unconsumed feed funnelled down the collecting bottle was collected 30 min after feeding, and was dried and weighed. Feed wastage was expressed as percentage of feed supplied. N loss to W was then estimated by multiplying the N content of the feed and percentage of feed wastage. The mean body weight of fish in each replicate cage was estimated at the beginning of the experiment and then monthly, by weighing thirty individual fish randomly sampled from each cage (after anaesthetising the fish in 100 ppm quinaldine for 1 to 2 min). Mortality of fish in each cage was also recorded monthly, and the biomass of survivors in each cage calculated each month. Production (*P*) was estimated by summing the monthly increase in biomass over the 1 yr study period:

$$\text{Production} = \sum_{t=0}^{12}[(N_t + N_{t+1})/2](W_{t+1} - W_t) \qquad (6)$$

where N_t and N_{t+1} are the number of survivors at time t and time $t + 1$, respectively; W_t and W_{t+1} are the mean body weights of fish at time t and time $t + 1$, respectively; t is the time in months ($t = 0$ to 12 mo). N channelled to *P* over the culture period was determined by multiplying the total biomass gain over period and the nitrogen content in the fish carcasses. Biomass loss to mortality in each cage was estimated using the following equation:

$$\text{Mortality} = \sum_{t=0}^{12}(N_t - N_{t+1})(W_t + W_{t+1})/2 \qquad (7)$$

and N loss via mortality (*M*) was then calculated from total biomass lost to mortality and nitrogen content in the fish carcasses.

Daily postprandial nitrogen excretion rates (A_t, mg N kg^{-1} d^{-1}) of the grouper stock in the sea cages were estimated from the daily water temperature and daily feed ration, using the following multiple regression equation derived for this species by Leung et al. (1999):

$$A_t = 22.81\,Temp_t + 28.78R_t - 378.18 \qquad (8)$$

where $Temp_t$ is the water temperature at Day t (in °C); and R_t is the feed ration size at Day t (in % body wt d^{-1}). Total N loss through ammonia excretion over the 1 yr culture period (*E*, in mg N) from each cage was then calculated by integrating daily ammonia excretion rates over the 1 yr period.

Results of our earlier laboratory experiments showed no significant differences between faecal production rates of *Epinephelus areolatus* under different water temperatures (ANOVA, $p > 0.05$). As a result, the overall average value of faecal N loss rate (35.16 ± 9.39 mg N kg^{-1} d^{-1}) derived from our laboratory experiments was used to estimate the N loss to daily faecal production (F) in the field.

Construction of laboratory and field N budgets. For the laboratory N budget, all budget items were expressed as either (1) g N fish^{-1} d^{-1} or (2) g N kg^{-1} of fish production. For the annual field budget, all budget items were expressed as g N m^{-3} yr^{-1} and g N kg^{-1} production yr^{-1}, respectively. The budget items were also expressed in terms of a percentage of the N input derived from: (1) feeding experiments and (2) summation of *W*, *P*, *M*, *E*, and *F*.

RESULTS

Laboratory N budget of individual *Epinephelus areolatus*

Feed wastage (*W*) of *Epinephelus areolatus* accounted for 16.0 ± 4.8% (mean ± SD) of total N input. The major forms of the nitrogen loss via feed wastage were organic nitrogen (96%), followed by ammonia (4%).

Nitrogen content in the carcasses of *Epinephelus areolatus* constituted 10.99% of dry weight. Production of *E. areolatus* was 15.1 g fish^{-1} mo^{-1} (equivalent to 1.67 g N fish^{-1} mo^{-1}). On average, N loss to feed wastage was 16% while the feed conversion ratio (FCR) was 6.52 (Table 1). The low FCR was partly attributed to the high water content (70%) of trash fish.

Table 1. Summary statistics on feed supplied, feed consumed, feed wastage, production, tissue nitrogen content, feed conversion, and feed efficiency for individual *Epinephelus areolatus* cultured under laboratory conditions for 1 mo at 25°C and 30‰ salinity

Parameter	Mean ± SD
Feed supplied (g fish^{-1} mo^{-1})	98.5 ± 8.8
Feed consumed (g fish^{-1} mo^{-1})	82.7 ± 7.4
Feed wastage (%)	16.0 ± 4.8
Production (g fish^{-1} mo^{-1})	15.1 ± 4.9
N content in tissue after culture period (% dry body wt)	10.99 ± 0.45
Feed conversion[a]	6.52
Feed efficiency[b]	0.15

[a] Feed conversion = feed supplied/production

[b] Feed efficiency = production/feed supplied

Table 2. Ammonia excretion rate, urea excretion rate, total organic nitrogen (TON) excretion rate, faecal production rate and faecal nitrogen production rate of *Epinephelus areolatus* within 24 h after feeding with trash fish (25°C and 30‰ salinity; ND = not detected)

Parameter	Mean ± SEM
Ammonia excretion (mg N kg^{-1} body wt d^{-1})	375.7 ± 50.3
Urea excretion (mg N kg^{-1} body wt d^{-1})	ND
TON excretion (mg N kg^{-1} body wt d^{-1})	ND
Faecal production (mg dry faeces kg^{-1} body wt d^{-1})	2730.9 ± 385.1
Faecal N production (mg N kg^{-1} body wt d^{-1})	47.2 ± 7.0

Urea and organic nitrogen were not detected in the excreta, suggesting that *E. areolatus* is ammoniotelic. Total ammonia-N (TAN) excretion rate of *E. areolatus* within 24 h after feeding was 375.7 mg N kg^{-1} d^{-1} while the faecal N production rate was 47.2 mg N kg^{-1} d^{-1} (Table 2).

Based on the above data, a laboratory N budget was constructed for individual *Epinephelus areolatus* (Table 3). The consumption value derived from the summation method (C_s) was 33.6% lower than the actual value determined in the feeding experiment (C_F). The majority of nitrogen consumed was excreted in the form of ammonia (63.9%) and via loss in faeces (8%). Only 28.1% of N consumed was channelled to growth (Table 3). N assimilation efficiency was 91.9%, while net N retention efficiency was 29.9%. Based on data given in Table 3, values of C_S, C_F, P, E and F for the production of 1 kg of fish is calculated (Table 4). Nitrogen loss through excretion and faecal production was 92.3 ± 10.1 g N kg^{-1} fish production, and accounted for 73.7% of total N consumed.

Field N budget for *Epinephelus areolatus* cultured in open-sea cages

Changes in temperature, salinity and dissolved oxygen at the study site during the experimental period are shown in Fig. 1. W of *Epinephelus areolatus* cultured in open-sea cages was 38 ± 8% (mean ± SD). Weight loss and reduction of total biomass were observed from February to March 1995 (Fig. 2a,c), when water temperature was low (16 to 18°C). Cumulative mortality of the culture stock was 32% over the 1 yr study period (Fig. 2b), and nitrogen content in the carcasses of *E. areolatus* at the end of the year was 11.5 ± 0.6%. Nitrogen channelled into P was estimated at 82.4 g N m^{-3} yr^{-1}, while N loss to mortality was 35.3 g N m^{-3} yr^{-1}.

Monthly values and cumulative values of each budget item are shown in Figs. 3 & 4, respectively. Overall consumption determined by the summation method (C_s) was 52% lower than the respective value estimated by the feeding experiment (C_F). All budget items (except F and M) decreased during February 1995 to April 1995 (Fig. 3), when negative production (P), negative net N retention efficiency (K_2) and poor budget balance were observed.

The annual nitrogen budget for open-sea-cage culture derived from the summation of monthly budgets is presented in Fig. 5. Assuming that C_F = 100% (Fig. 5a), then 32.4% of input N could not be accounted for, and the importance of budget items followed the decreasing order: $W > E >> P > F > M$. Assuming that C_S = 100% (Fig. 5b), the value of the budget items followed the order: $E > W >> P > F > M$. The most important budget items contributing to N pollution loading were E (46.0% of total N input), followed by W (37.7% of total N input) (Fig. 5b). F and M together contributed less than 8% of total N input; while P accounted for less than 9% of total N input. The overall nitrogen assimilation efficiency of trash fish by *Epinephelus areolatus* was 94%, while the net nitrogen retention efficiency was 15%.

Based on the N budget of *Epinephelus areolatus* in open-sea cages (Fig. 5b), values of the budget items for production of 1 kg of fish were also derived (Table 5). Total N loss through feed wastage, excretion and faecal production was 320.6 g N kg^{-1} fish

Table 3. Laboratory N budget for individual *Epinephelus areolatus* (n = 8), cultured at 25°C and 30‰ salinity for a month. All budget items were measured independently and expressed in mg N $fish^{-1}$ d^{-1} (mean ± SEM). C_S = consumption derived from summation method; C_F = consumption derived from feeding experiments. Percentage of budget's balance = $(C_S/C_F) \times 100\%$ nitrogen assimilation efficiency = $[(C_S - F)/C_S] \times 100\%$, net nitrogen retention efficiency = $[P/(C_S - F)] \times 100\%$, P = production, E = excretion, and F = faecal production

	P (1)	E (2)	F (3)	C_S (1+2+3)	C_F	% Balance = C_S/C_F	P/C_S (%)	E/C_S (%)	F/C_S (%)	$(C_S-F)/C_S$ (%)	$P/(C_S-F)$ (%)
mg N $fish^{-1}$ d^{-1}	16.6 ± 1.9	37.7 ± 1.5	4.7 ± 0.2	59.0 ± 3.4	88.6 ± 2.6	66.4 ± 2.6	27.5	64.4	8.1	91.9	29.9
%	28.1	63.9	8.0	100							

Table 4. Nitrogen budget of individual *Epinephelus areolatus* (n = 8), cultured at 25°C and 30‰ salinity under laboratory condition for a month. Data are presented as a mass balance for N from the production of 1 kg of fish and given as g N kg^{-1} fish

P	E	F	C_S	C_F	N loss ($F + E$)	% N loss $(E + F)/C_S$
33.0 ± 0.1	82.0 ± 10.0	10.3 ± 1.3	125.3 ± 11.3	193.7 ± 23.5	92.3 ± 10.1	73.7

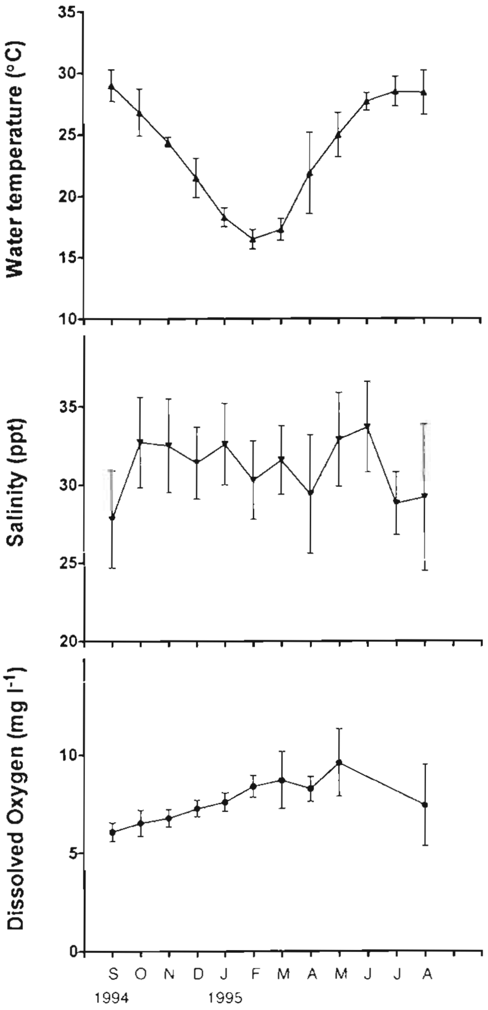

Fig. 1. Water temperature, salinity and dissolved oxygen at the open-sea-cage farm during August 1994 to August 1995. Data are expressed in mean ± SD of the month

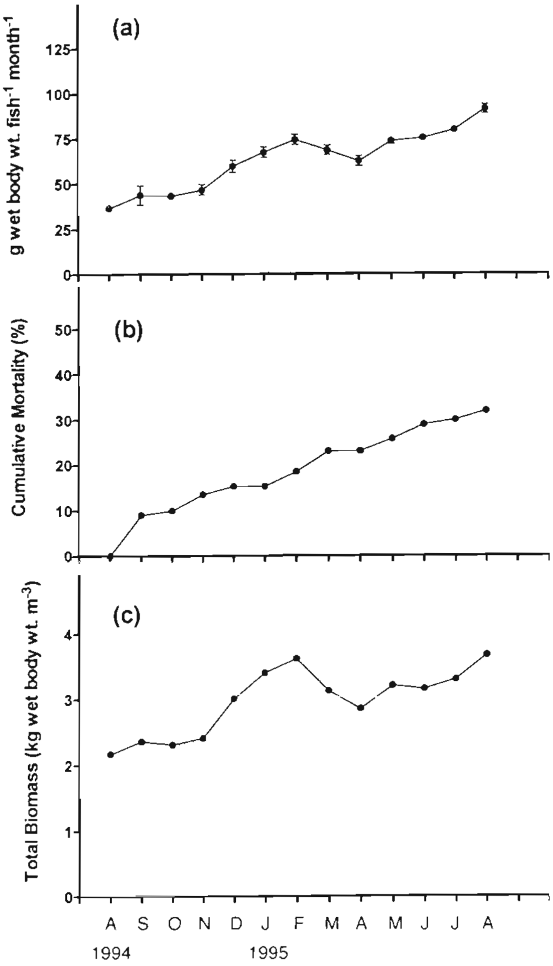

Fig. 2. (a) Mean body weight; (b) cumulative mortality; and (c) total biomass of *Epinephelus areolatus* cultured in an open-sea-cage farm during August 1994 to August 1995

production, and accounted for 87.7% of total N input. N loss was predominantly in the form of ammonia, followed by organic N (Table 6).

DISCUSSION

Laboratory N budget of individual *Epinephelus areolatus*

The nitrogen content of *Epinephelus areolatus* (10.99% dry wt) is similar to that reported for other tropical/sub-tropical culture species (e.g. 11.67% for *E. salmonides*, 9.82% for *E. tauvina* and 10.80% for *Lates calcarifer*; Chen et al. 1987, Tacon et al. 1991). Ammonia excretion was the most important item (64% of total N consumed) in the nitrogen budget of *E. areolatus*. Production (28% of N consumed) was the second most important budget item. N loss to faecal production constituted only 8% of N consumed. Such an order of importance (i.e. Excretion >> Growth > Faecal Production) was also generally observed in studies on certain temperate species

Table 5. Nitrogen budget for *Epinephelus areolatus* cultured in an open-sea-cage farm

Budget item	g N kg^{-1} fish production
Input (*I*)	365.5
Wastage (*W*)	138.0
Production (*P*)	31.5
Excretion (*E*)	168.2
Faeces (*F*)	14.4
Mortality (*M*)	13.5
N loading to culture water (*W* + *E* + *F*)	320.6

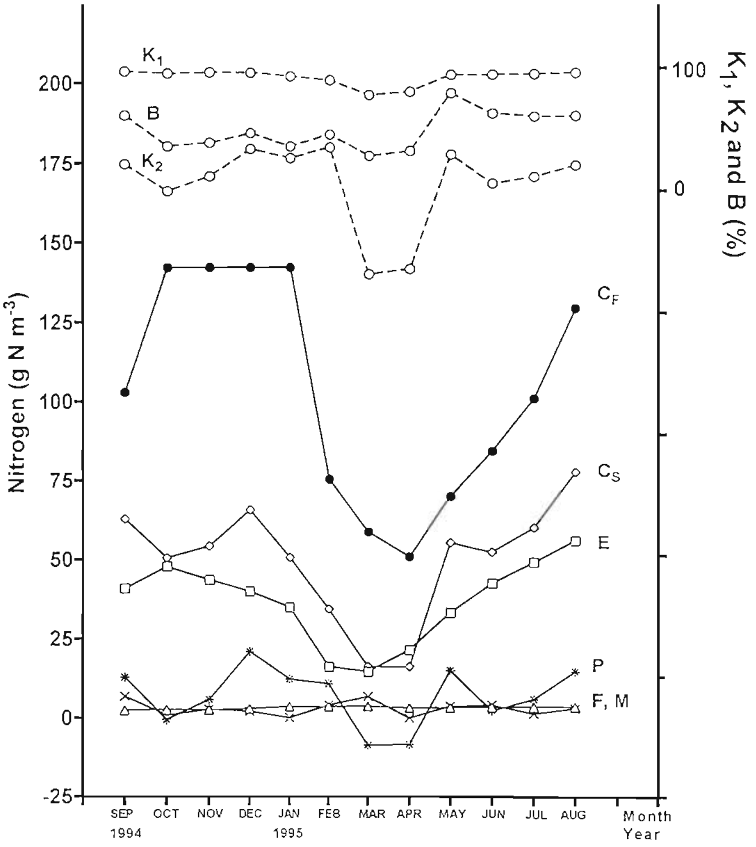

Fig. 3. Monthly values of various budget items of *Epinephelus areolatus* cultured in an open-sea-cage farm. See Table 5 for definitions of budget items

(Table 7). Carter & Brafield (1992), however, found that N channelled to growth (69% of N consumed) was much higher than excretion (26%) in the grass carp *Ctenopharyngodon idella* (Table 7). It has been demonstrated that budget items may be affected by species, diet, feeding regimes, physiological characteristics and growth performance of the fish as well as experimental protocols between studies (Handy & Poxton 1993, Heinsbrock et al. 1993).

Table 6. Various forms of nitrogen pollution loading (g N kg production^{-1}) of *Epinephelus areolatus* cultured in the open-sea-cage farm. Forms of N are also shown as percentage of total N (TN) loss. (ND = not detected)

	N pollution loading	
	g N kg^{-1} production	% of TN loss
Total ammonia N	169.8	53.0
Nitrate N	0.7	0.2
Nitrite N	0.1	0.0
Urea N	ND	ND
Total organic N (TON)	150.0	46.8
Total dissolved N (TDN)	170.6	53.2
Total N	320.6	100.00

Heinsbrock et al. (1993) showed that the N budgets of freshwater eel *Anguilla anguilla* and carp *Cyprinus carpio* accounted for 68 to 96% of the actual N intake. In the present study, the N budget of individual *Epinephelus areolatus* only accounted for 66.4 ± 2.6% (mean ± SEM) of actual nitrogen intake. Thus, some 31 to 36% of the nitrogen intake was unaccounted for in the present laboratory budget. Imbalance of N budgets has been commonly reported in other studies, and attributed to excretion of urea and dissolved organic N (DON) (Handy & Poxton 1993, Heinsbrock et al. 1993). However, neither urea nor DON was detected in the excretion of *E. areolatus*. Indeed, urea and DON were not detected in many other species (e.g. Porter et al. 1987, Heinsbrock et al. 1993). For those studies in which urea and DON were reported to be significant, measurements were conducted in a static system over a prolonged period (e.g. 8 h or more). It is likely that the build-up of fish excretory products in a static system may affect the physiological response, thereby changing the pattern of excretion of the experimental fish (Wood 1993). For example, it has been shown that high levels of ammonia (25 mM NH_4Cl) may shift ammoniotelism to ureotelism in *Heteropneuates fossilis* in order to detoxify ammonia (Saha & Ratha 1986, 1990). The use of a flow through system in the present experiment prevented the build-up of ammonia, and probably explained the absence of urea and DON in the excretory products of *E. areolatus*.

The imbalance of the budget in the present study may be attributed to the cumulative experimental errors incurred in the determination of individual budget items. Excretion of ammonia will increase during activities (e.g. swimming and foraging) because protein may be catabolised during exercise (Sukumaran & Kutty 1977). In the present study, ammonia excretion was only measured under basal metabolic conditions since the experimental fish were confined to an experimental chamber. As a result, ammonia excretion might have been underestimated, and this partially explains the imbalance of the current N budgets. The standard deviation of W measured in the laboratory was about ±5% of input N; estimates of C_F would therefore also be subject to an experimental error. The cumulative error in the above estimations might therefore account for the 32 to 34% imbalance in the N budget.

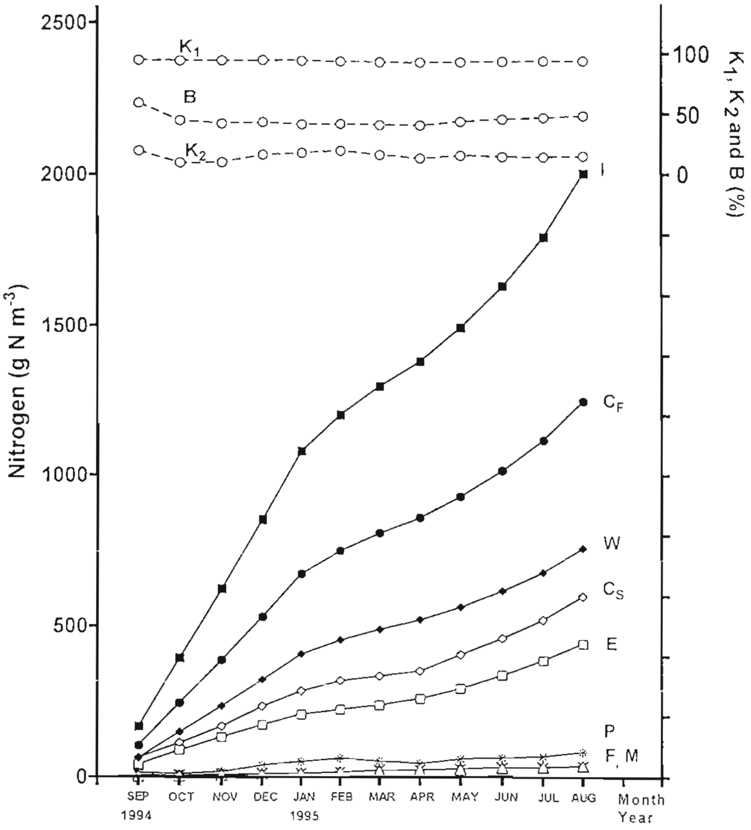

Fig. 4. Cumulative values of various budget items of *Epinephelus areolatus* cultured in an open-sea-cage farm. See Table 5 for definitions of budget items.

Field N budget for *Epinephelus areolatus* cultured in open-sea cages

Some 19 to 28% of total nitrogen input into a trout farm could be harvested in the form of fish production (Penczak et al. 1982, Gowen et al. 1985, Philips et al. 1985, Persson 1986, Enell 1987, Foy & Rosell 1991, Hall et al. 1992). Compared with trout farms, the percentage of N input that can be harvested from open cage cultures of *Epinephelus areolatus* (8.6%) was very low. The poor growth performance of *E. areolatus* found in the current study may be partially attributed to their negative growth and reduction of total biomass during cold winter months (i.e. February and March 1995), which is typical in Hong Kong. Another important contributing factor was the high feed wastage (30 to 46% of N input) in open-sea-cage culture. Despite the high cumulative mortality (32%) encountered, the contribution of fish mortality to the overall N budget was, however, relatively small (1.8%). Similarly, Hall et al. (1992) found that only 2 to 5% of N input was lost through mortality in rainbow trout cultured in open-sea cages in Sweden. In the present study, 88% of the total nitrogen input to the *E. areolatus* culture farm was lost to the environment, and such a loss falls within the range (52 to 95%) reported by Handy & Poxton (1993). The N

Table 7. A comparison on the nitrogen budget of different fish species reported in the literature. Data expressed as percentage of N consumed. *Budget item was derived from the difference of budget items in the mass balance equation: $C = P + E + F$; C = consumption, P = production, E = excretion and F = faecal production

Species (initial mean body wt is given, if known)	C	P	E	F	$(C-F)/C$ (%)	$P/(C-F)$ (%)	Source
Areolated grouper *Epinephelus areolatus* (93 g)	100*	25.6	66.1	8.3	91.7	27.9	This study
Gilthead seabream *Sparus aurata* (90 g)	100*	21.3	73.0	5.7	94.3	22.6	Porter et al. (1987)
Rainbow trout *Oncorhynchus mykiss* (35 g)	100	33.0	54.6*	12.4	87.6	37.7	Asgard et al. (1991)
Red tilapia *Sarotherodon* sp. (75 g, stocking density: 50 m^{-3})	100	19.4	61.7*	18.9	81.1	23.9	Suresh & Lin (1992)
Grass carp *Ctenopharyngodon idella*	100	69.2*	25.9	4.9	95.1	72.8	Carter & Brafield (1992)
Rainbow trout *Salmo gairdneri*	100	22.0	78.0*		–	–	Gowen & Bradbury (1987)
Rainbow trout *Oncorhynchus mykiss*	100	25.0	60.0*	15.0	85.0	29.4	Hakanson et al. (1988)

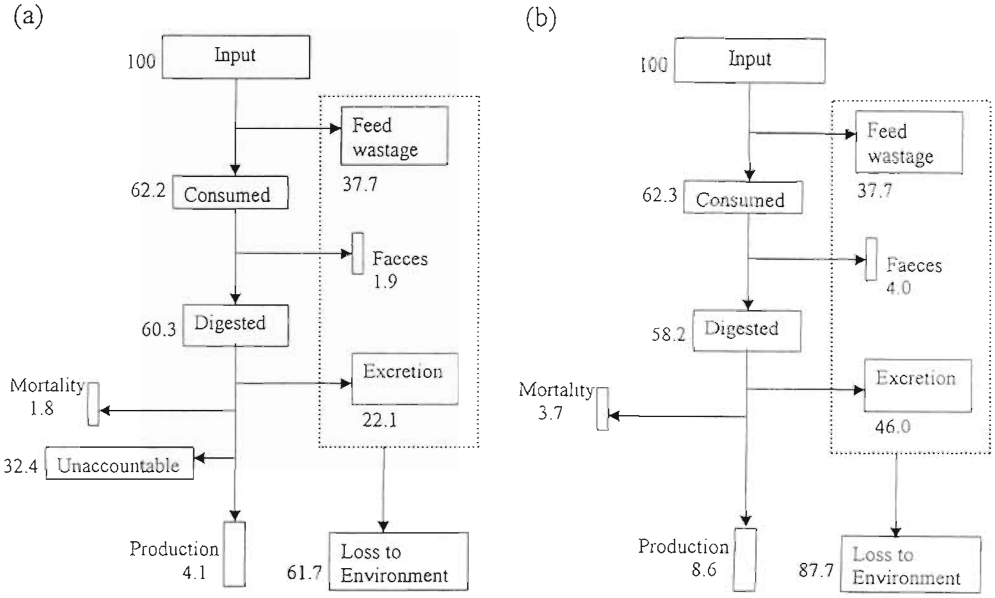

Fig. 5. Annual N budgets of *Epinephelus areolatus* cultured in an open-sea-cage farm. (a) Assuming that C_F = 100%; and (b) assuming that C_S = 100%

loading from salmonid cage farms ranged from 73.3 to 124.2 kg N t^{-1} (Sumari 1982, Warren-Hansen 1982, Gowen et al. 1985, Enell 1987, Foy & Rosell 1991, Hall et al. 1992). The total environmental loss from *E. areolatus* cage cultures was 321 kg N t^{-1} of fish production. Thus, *E. areolatus* farming using trash fish has a much higher N loading per kg production than that of culturing salmonid species, and correspondingly, a greater environmental impact may be expected.

The annual production of marine culture fish in Hong Kong is around 3000 t (equivalent to 99 t N), and more than 10 species of groupers (Serranidae), seabreams (Sparidae) and snappers (Lutjanidae) are commonly culutured. By (1) assuming the production of nitrogen wastes for the other culture species is similar to that of *Epinephelus aerolatus*, and (2) applying the nitrogen budget derived for open-sea cages (Table 5), the total nitrogen waste generated by the industry is estimated at 962 t N yr^{-1} (of which some 510 t yr^{-1} is in form of ammonical-N). Such a high value shows that the marine fish farming industry contributes to a significant nitrogen loading into the coastal waters of Hong Kong.

Sources contributing to the N loss from fish farms include feed wastage, faecal production and excretion. In this study, ammonia excretion of *Epinephelus areolatus* was clearly the major source of N loss, followed by feed wastage. Faecal N production was negligible, and contributed less than 4% of total N input to the culture system. A similar pattern of N loss from open-sea-cage farms was observed in salmonid culture in temperate regions (Table 8), although feed wastage of *E. areolatus* fed with trash fish (30 to 46% of N input) was much higher than that of rainbow trout fed with pellets (20 to 23% of N input; Gowen et al. 1985, Hall et al. 1992).

The present field N budget for *Epinephelus areolatus* clearly showed that ammonical-N was the major form of nitrogen loss to the environment, followed by total organic nitrogen (TON). In general, the amount of dissolved nitrogen, including ammonia and urea from fish excreta, was higher than that of TON (Table 9; for a review, see Handy & Poxton 1993). This implies that most N released from the farm will be lost to the water column rather than to bottom sediment, and may therefore affect a wider area (Wu et al. 1994).

The value of N consumed estimated from the summation method (C_S) was 52% lower than that derived from the feeding experiment.% balance would be expected to be much lower for the N budget constructed for fish farm in natural, variable, field conditions as compared with laboratory studies of individual fish (66.5% of C_F).

Table 8. A comparison between the nitrogen budget of the areolated grouper *Epinephelus areolatus* and rainbow trout *Oncorhynchus mykiss* cultured in open-sea-cage farms (data are expressed as % of total N input into the farm). *W* = wastage, *F* = faecal production, *E* = excretion, *P* = production, *M* = mortality

Species	*W*	*F*	*E*	*P*	*M*	Source
E. areolatus	37.7	4.0	46.0	8.6	3.7	This study
O. mykiss		23.0	48.0	27.0–28.0	2.0–5.0	Hall et al. (1992)
		74.0		26.0		Enell (1987)
		72.0		28.0		Persson (1986)
		79.0		21.0		Phillips et al. (1985)
	20.0	8.0	52.0	20.0		Gowen et al. (1985)
		78.5		21.5		Penczak et al. (1982)

Table 9. A comparison between N loss in rainbow trout and grouper open-sea-cage farms (data are expressed as % of total N input into the farm). TDN = total dissolved nitrogen including ammonia and urea from fish excreta; TON = total organic nitrogen from feed wastage and faecal production

Species	Loss of TDN to water	Loss of TON to sediment	Total N loss	Total N loss (kg N t^{-1} production)	Source
Epinephelus areolatus	47	41	88	321	This study
Oncorhynchus mykiss	52	28	80	100	Gowen et al. (1985)
Oncorhynchus mykiss	58	16	74	86	Enell (1987)
Oncorhynchus mykiss	48	23	71	102	Hall et al. (1992)

The most significant error in the field budget is likely to have resulted from the estimation of *in situ* feed wastage. In this study, errors of feed wastage were found to be 8% of total N input. Second, notwithstanding the error derived from the laboratory measurement of *E* and *F* mentioned previously, the physiological responses (and hence *E* and *F*) of fish in open-sea cages may be different from fish cultured under laboratory conditions (Handy & Poxton 1993). Also, extrapolating the data derived from the laboratory experiment to fish cultured in open-sea cages may be subject to day-to-day and seasonal variations in physical parameters, and may hence incur an error in estimating *E* and *F* of fish farms. Nevertheless, *in situ* measurements of *E* and *F* are considered to be impractical. Third, estimation of various budget items was based on mean size of fish. Size variation of fish in each experimental cage was, however, considerable. For example, the coefficient of variability of the weight of *Epinephelus areolatus* increased from 24.1 to 49.3% throughout the study period. Brannas & Alanara (1993) suggested that large size or energetic and aggressive individuals may consume more feed in a cage because of the dominant hierarchy behaviour in a school of fish, leading to under-feeding and slow growth of less aggressive individuals. Size variation indicates that physiological conditions and foraging efficiency of individual fish may be different within a single replicate and the use of average body weight and mortality to estimate *E*, *F*, *P* and *M* may incur a potential error. Despite the above practical limitations and experimental errors, the present nitrogen budgets constructed under laboratory and field conditions provide a general picture and a model of N-utilisation in open-sea-cage farms.

The present nitrogen budgets constructed for *Epinephelus areolatus* show that N losses from sub-tropical open-sea-cage farms using trash fish as fish feed are almost 3 times higher than those in temperate salmonid farms. Such a high N loading can be reduced by (1) replacing the use of trash fish with an artificial nutritional-balanced diet (with a minimum N content to support optimal growth), which could improve FCR and reduce feed wastage; and (2) utilising or harvesting the released N by integrated culture with bivalves and macroalgae (Wu 1995).

Acknowledgements. Part of this work received technical and financial support from the Department of Agriculture and Fisheries of the Hong Kong Government. We would like to thank Dr P Wong, A Kowk and staff of the Kat O Fisheries Research Station for their support, technical assistance and invaluable advice throughout this project.

LITERATURE CITED

Ackefors H (1986) The impact on the environment by cage farming in open water. J Aquacult Trop 1:25–33

Ackefors H, Enell M (1990) Discharge of nutrients from Swedish fish farming to adjacent sea areas. Ambio 19:28–35

Asgard T, Langaker RM, Shearer KD, Austreng E, Kittelsen (1991) Ration optimization to reduce potential pollutants—preliminary results. Am Fisheries Soc Symp 10:410–416

Aure J, Stigebrandt A (1990) Quantitative estimates of the eutrophication effects of fish farming on fjords. Aquaculture 90:135–156

BC Ministry of Environment (1990) Environmental management of marine fish farms. British Columbia Ministry of Environment, Victoria

Brannas E, Alanara A (1993) Monitoring the feeding activity of individual fish with a demand feeding system. J Fish Biol 42:209–215

Carter CG, Brafield AE (1992) The bioenergetics of grass carp, *Ctenopharyngodon idella* (Val.): the influence of body weight, ration and dietary composition on nitrogenous excretion. J Fish Biol 41:533–543

Chen TF, Liu CY, Lin KJ (1987) The experiment for development of artificial diet for salmon-like grouper *Epinephelus salmonoides*—experiment of the nutrition requirement and rearing study by feeding with artificial diet. Bull Taiwan Fish Res Inst 43:301–317

Chu JCW, Leung KMY, Wu RSS (1995) Effect of feed types on feed wastage of areolated grouper (*Epinephelus areolatus*) cultured in open water cages. In: Chou LM, Munro AD, Lam TJ, Chen TW, Cheong LKK, Ding JK, Hooi KK, Khoo HW, Phang VPE, Shim KF, Tan CH (eds) Abstracts of the Fourth Asian Fisheries Forum, Beijing. The Asian Fish Soc, Manila

Cockcroft AC, McLachlan A (1993) Nitrogen budget for a high-energy ecosystem. Mar Ecol Prog Ser 100:287–299

Duff A (1987) Scottish fish farm pollution. Mar Pollut Bull 18:261

Dugdale RC (1967) Nutrient limitation in the sea: dynamics, identification and significance. Limnol Oceanogr 12: 685–695

Eloranta P, Palomaeki A (1986) Phytoplankton in Lake Konnevesi with special reference to eutrophication of the lake by fish farming. Aqua Fenn 16:37–45

Enell M (1987) Environmental impact of cage fish farming with special reference to phosphorus and nitrogen loadings. Comm Meet Int Counc Explor Sea, CM–ICES/F:44, Ref. MEQC

FAO (1995) Aquaculture production statistics, 1984–1993. Fisheries circular no. 815, Revision 7 Fisheries and Agriculture Organization, Rome

Foy RH, Rosell R (1991) Loadings of nitrogen and phosphorus from a Northern Ireland fish farm. Aquaculture 96: 17–30

Gowen RJ, Bradbury NB (1987) The ecological impact of salmonid farming in coastal waters: a review. In: Barnes M (ed) Oceanography and marine biology: an annual review. Oceanogr Mar Biol Annu Rev 25:563–75

Gowen RJ, Bradbury NB, Brown JR (1985) The ecological impact of salmon farming in Scottish coastal waters: a preliminary appraisal. Comm Meet Int Counc Explor Sea, CM–ICES/F:35, Ref. E+C, Sess W

Gundersen K (1981) The distribution and biological transformation of nitrogen in the Baltic Sea. Mar Pollut Bull 12:199–205

Hakanson L, Ervik A, Makinen T, Moller B (1988) Basic concepts concerning assessments of environmental effects of marine fish farms. Report Nord 1988:90. Nordic Council of Ministers, Copenhagen

Hall POJ, Holby O, Kollberg S, Samuelsson M (1992) Chemical fluxes and mass balances in a marine fish cage farm. IV. Nitrogen. Mar Ecol Prog Ser 89:81–91

Hammo LS (1987) Mariculture pollution. Mar Pollut Bull 18:148

Handy RD, Poxton MG (1993) Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Rev Fish Biol Fish 3:205–241

Heinsbrock LTN, Tijssen PAT, Flach RB, De-Jong GDC (1993) Energy and nitrogen balance studies in fish. In: Kanshik SJ, Liquet P (eds) Fish nutrition in practice. Proc 4th Int Symp Fish Nutr Feed, Biarritz 24–27 June 1991, Institut National de la Recherche Agronomique, Paris, France, no. 61, p 375–389

Jones KJ, Ayres P, Bullock AM, Roberts RJ, Tett P (1982) A red tide of *Gyrodinium aureolum* in sea lochs of the Firth of Clyde and associated mortality of pond-reared salmon. J Mar Biol Assoc UK 62:771–782

Leung KMY, Chu JCW, Wu RSS (1999) Effects of body weight, water temperature and ration size on ammonia excretion by areolated grouper *Epinephelus areolatus* and mangrove snapper *Lutjanus argentimaculatus*. Aquaculture 170:215–227

New MB, Csavas I (1995) Aquafeed in Asia—a regional review. Farm made aquafeed. FAO Fisheries Technical paper no. 343

Penczak T, Galicka W, Molinski M, Kusto E, Zalewski M (1982) The enrichment of a mesotrophic lake by carbon, phosphorus and nitrogen from the cage aquaculture of rainbow trout, *Salmo gairdneri*. J Appl Ecol 19:371–393

Persson G (1986) Kassodling av regnbage; Narsaltemissioner och miljo vid tre odlingslagen langs Smalandskusten. Report 3215. National Swedish Environmental Protection Board, Solna

Phillips MJ, Beveridge, MCM, Ross LG (1985) The environmental impact of salmonid cage culture on inland fisheries: present status and future trends. J Fish Biol (Suppl A)27:123–127

Porter CB, Krom MD, Robbins MG, Brickell L, Davidson A (1987) Ammonia excretion and total N budget for gilthead seabream (*Sparus aurata*) and its effect on water quality conditions. Aquaculture 66:287–297

Rosenthal H, Weston D, Gowen R, Black E (1988) Report of the *ad hoc* study group on Environmental Impact of Mariculture. ICES Coop Res Rep 154

Saha N, Ratha K (1986) Effect of ammonia stress on ureogensis in a freshwater air-breathing teleost, *Heteropneustes fossils*. In: Kon OL, Chung MCM, Hwong PLH, Leong SF, Loke KH, Thiyagarh P, Wong PTH (eds) Contemporary themes in biochemstry, Vol 6. Cambridge University Press, Cambridge, p 342–343

Saha N, Ratha BK (1990) Alternation in excretion pattern of ammonia and urea in a freshwater air-breathing teleost, *Heteropneustes fossilis* (Bloch) during hyper-ammonia stress. Indian J Exp Biol 28:597

Skjoldal HE, Dundas I (1991) The *Chrysochromulina polylepis* in the Skagerrak and the Kattegat in May—June 1988. Environmental conditions, possible causes, and effects. ICES Coop Res Rep 175

Skogheim OK, Bremnes K (1984) Effect on the environment from fish farms in lakes. Norsk Fiskeoppdrett 9:37–38

Soley N, Neiland A, Nowell D (1994) An economic approach to pollution control in aquaculture. Mar Pollut Bull 28:170–177

Strickland JD, Parsons TR (1972) A practical handbook of seawater analysis. Bull Fish Res Board Can 167:310

Sukumaran N, Kutty MN (1977) Oxygen consumption and ammonia excretion in the catfish *Mystus armatus*, with specific reference to swimming speed and ambient oxygen. Proc Indian Acad Sci Section B 86:195–206

Sumari O (1982) A report on fish farm effluents in Finland. In: Alabaster JS (ed) Report of the EIFAC Workshop on Fish Farm Effluents, Silkeborg, Demark, 26–28 May 1981. EIFAC Tech. Paper 41:29–55

Suresh AV, Lin CK (1992) Effect of stocking density on water quality and production of red tilapia in a recirculated water system. Aquacult Eng 11:1–22

Tacon AGJ, Rausin N, Kadari M, Cornelis P (1991) The food and feeding of tropical marine fishes in floating net cages: Asian seabass, *Lates calcarifer* (Bloch), and brown-spotted grouper, *Epinephelus tauvina* (Forskal). Aquacult Fish Manage 22:165–182

Waldichuk M (1987) Fish farming problems. Mar Pollut Bull 18:2–3

Warrer-Hansen I (1982) Methods of treatment of waste water from trout farming. In: Alabaster JS (ed) Report of the EIFAC Workshop on Fish-Farm Effluents, Denmark, May 1981. EIFAC Tech Paper 41:113–21

Wildish DJ, Martin JL, Wilson AJ, Ringuette M (1990) Environmental monitoring of the Bay of Fundy salmonid mariculture industry during 1988–89. Can Tech Rep Fish Aquat Sci No. 1760

Wood CM (1993) Ammonia and urea metabolism and excretion. In: Kennish MJ, Lutz PL (eds) The physiology of fishes. CRC Press, Florida, p 379–425

Wu RSS (1988) Marine pollution in Hong Kong: a review. Asian Mar Biol 5:1–23

Wu RSS (1995) The environmental impact of marine fish culture: towards a sustainable future. Mar Pollut Bull 31: 159–166

Wu RSS, Lam KS, Mackay DW, Lau TC, Yam V (1994) Impact of marine fish farming on water quality and bottom sediment: a case study in the sub-tropical environment. Mar Environ Res 38:115–145

Editorial responsibility: Charles Birkeland (Contributing Editor), Mangilao, Guam

Submitted: January 29, 1998; Accepted: May 5, 1999
Proofs received from author(s): September 6, 1999