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Dietary sodium requirement determined for juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) reared in fresh water and seawater

Shi-Yen Shiau* and Li-Shan Lu

Department of Food Science, National Taiwan Ocean University, Keelung 202, Taiwan

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Two 8-week feeding trials were conducted to determine the dietary Na requirement for juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) reared in fresh water and seawater. In each experiment, NaCl was added to the basal diet at 0, 0·5, 1, 2, 3, 5, or 7 g Na/kg diet (fresh water) and at 0, 0·2, 0·5, 0·8, 1·2, 1·5, 2, or 3 g Na/kg diet (seawater). Each diet was fed to three replicate groups of fish, individual fish initially weighing 0·69 (SE 0·01) g, in a closed, recirculating rearing system. In fresh water, the tilapia fed the diet supplemented with 2 g Na/kg diet had significantly ($P<0·05$) greater weight gain than the fish fed the diets supplemented with ≥3 and ≤0·5 g Na/kg diet. Feed efficiency (FE) in fish generally followed the weight-gain pattern. Gill Na^+-K^+ ATPase activity was highest in the fish fed the diets supplemented with 1–3 g Na/kg diet, followed by the fish fed the diet with 7 g Na/kg diet and lowest in the fish fed the unsupplemented control diet. In seawater, the weight gain, FE and gill Na^+-K^+ ATPase activity in fish were not affected by the dietary treatment. Analysis by polynomial regression of weight gain, by broken-line regression of gill Na^+-K^+ ATPase activity and by linear regression of whole-body Na retention of the fish reared in fresh water, indicated that the adequate dietary Na concentration for tilapia is about 1·5 g/kg diet. The present study also suggests that no dietary Na is required for tilapia reared in seawater.

Sodium: Fish: Tilapia

Tilapia are mainly lacustrine fish and are well adapted to enclosed water from low salinity (fresh water) to high salinity (seawater). They are widely cultured in tropical and subtropical regions of the world and constitute the third largest group of farmed finfish, with an annual production growth rate of about 11·5 % (El-Sayed, 1999), and a future increase in production has been projected (New, 1999).

Knowledge in the area of tilapia nutrition has increased greatly in recent years. There is a need for further advancements in nutritional research as tilapia production becomes more intensive. One class of nutrients which has not been extensively studied with respect to tilapia is the minerals (Shiau, 2002).

Na and K are essential minerals in animals because of their role in electrolyte and acid–base balance; Na^+ is the principal extracellular cation, whereas K^+ is the principal intracellular cation in animal tissues. Dietary requirements for these two minerals have been reported for several land animals. However, fish are known to readily exchange these minerals across their gills in order to maintain acid–base balance and osmotic pressure with their aquatic environment. The requirement for K in tilapia has recently been established (Shiau & Hsieh, 2001). The dietary requirement of Na in fish has only been qualitatively determined by supplementation with NaCl and the results vary. Dietary supplementation with NaCl improved the growth of common carp (*Cyprinus carpio* (Linn.)) and mrigal (*Cirrhinus mrigala* (Ham.)) (Nandeesha *et al.* 2000) and also the growth of red drum (*Sciaenops ocellatus*; Gatlin *et al.* 1992) in fresh-water environments. However, beneficial effects of NaCl supplementation on growth were not observed in Atlantic salmon (*Salmo salar*; Shaw *et al.* 1975) and rainbow trout (*Oncorhynchus mykiss* (MacLeod, 1978); *Salmo gairdneri* Richardson (Salman & Eddy, 1988).

The purpose of the present study was to estimate the dietary Na requirement of juvenile tilapia (*Oreochromis niloticus* × *O. aureus*) reared in fresh water and seawater using growth indices supported by the measurement of gill Na^+-K^+ ATPase activity and whole-body Na retention.

Materials and methods

Diet preparation

The experimental diet formulation is given in Table 1. The formulation, which has been shown to be adequate for tilapia, was similar to that used by Shiau & Lo (2000), except that the protein content of the diet was adjusted to the optimal level required for tilapia reared in seawater (Shiau & Huang, 1989). Vitamin-free casein (Sigma Chemical Co., St Louis, MO, USA) was used as the protein source. The mineral mixture was similar to that used by Shiau & Hsieh (2001), except that it did not contain Na.

* **Corresponding author:** Dr Shi-Yen Shiau, fax +886 2 2462 1684, email syshiau@mail.ntou.edu.tw

Table 1. Formulation of the basal diets (g/kg diet)

	Fresh water	Seawater
Ingredients		
Casein (vitamin free)	380	300
Maize starch	380	380
Maize oil	70	70
Fish oil	40	40
Vitamin mixture*	20	20
Mineral mixture†	40	40
Carboxymethylcellulose	20	20
α-Cellulose	50	130
Proximate composition		
Moisture	104	104
Crude protein	310	241
Diethyl ether extract	108	113
Crude fibre	35	116
Ash	34	32
N-free extract‡	409	394

* Vitamin mixture (mg/g mixture): thiamin hydrochloride, 5; riboflavin, 5; calcium pantothenate, 10; nicotinic acid, 6·05; d-biotin, 0·003; pyridoxine hydrochloride, 0·825; folic acid, 0·041; inositol, 200; L-ascorbyl-2-monophosphate-Mg, 2·025; choline chloride, 44; menadione, 4; α-tocopheryl acetate, 3·35; retinyl acetate, 0·4; *para*-aminobenzoic acid, 5; cholecalciferol, 0·0004685. All ingredients were diluted with α-cellulose to 1 g.

† Mineral mixture (mg/g mixture): $AlCl_3 \cdot 6H_2O$, 0·15; KI, 0·15; $CuSO_4$, 0·1; $MnSO_4 \cdot H_2O$, 0·8; $CoCl_2 \cdot 6H_2O$, 1; $ZnSO_4 \cdot 7H_2O$, 3; $Ca(H_2PO_4)_2 \cdot H_2O$, 135·8; $Ca(CH_3CHOHCOO)_2 \cdot 5H_2O$, 327; $FeSO_4 \cdot 6H_2O$, 2·125; $MgSO_4 \cdot 7H_2O$, 137; KCl, 75. All ingredients were diluted with α-cellulose to 1 g.

‡ N-free extract = 1000 − moisture − crude protein − diethyl ether extract − crude fibre − ash.

In the fresh-water experiment, the basal diet was supplemented with NaCl (Hayashi Pure Chemical Industries Ltd, Osaka, Japan). Supplementation was at 0, 0·5, 1, 2, 3, 5, or 7 g Na/kg dry diet. In the seawater (salinity 32–34 parts per trillion) experiment, the basal diet was supplemented at 0, 0·2, 0·5, 0·8, 1·2, 1·5, 2, or 3 g Na/kg diet. The Na concentrations of the fifteen diets were determined by flame photometry after dry ashing according to the method of the Association of Official Analytical Chemists (1995).

In the fresh-water experimental diets the Na concentrations were found to be (g/kg): unsupplemented control, 0·27; 0·5 g/kg supplementation, 0·72; 1 g/kg supplementation, 1·20; 2 g/kg supplementation, 2·24; 3 g/kg supplementation, 3·21; 5 g/kg supplementation, 5·28; 7 g/kg supplementation, 7·71. In the seawater experimental diets the Na concentrations were (g/kg): unsupplemented control, 0·21; 0·2 g/kg supplementation, 0·51; 0·5 g/kg supplementation, 0·75; 0·8 g/kg supplementation, 1·01; 1·2 g/kg supplementation, 1·35; 1·5 g/kg supplementation, 1·72; 2 g/kg supplementation, 2·24; 3 g/kg supplementation, 3·16.

The diets were prepared by thoroughly mixing the dry ingredients with oil and then adding cold water until a stiff dough resulted. This was then passed through a mincer with die and the resulting strings were dried using an electrical fan at 28°C. After drying, the diets were broken up, sieved into pellets and stored at −20°C.

Experimental procedure

Male hybrid tilapia were supplied from the Far East Hatchery (Cha-Yi, Taiwan). Upon arrival, they were acclimatised to laboratory conditions for 4 weeks in a plastic tank (0·74 m wide, 0·95 m long, 0·45 m high) and fed a commercial diet (Hung Kuo Industrial, Taipei, Taiwan). The culture system consisted of individual glass aquaria (0·305 m wide, 0·610 m long, 0·555 m high), each containing approximately 60 litres water and was a part of a closed recirculated system with a common water reservoir maintained at 26 ± 1°C. The water was circulated at 2 litres/min through two separate biofilters to remove impurities and reduce NH_3 concentrations. Two systems were used in the study; one was designated for the fresh-water experiment and one for the seawater experiment.

At the beginning of the experiment, fifteen fish (mean weight 0·69 (SE 0·01) g) were stocked in each aquarium. There were fifteen treatments. Each experimental diet was fed to fish in three aquaria. The fish chosen for the experiment and the diets were assigned to groups of fish randomly. The fish were fed 50 g diet/kg body weight per d. This amount was close to the maximum daily rations consumed by the tilapia during the acclimatisation period. The daily ration was subdivided into two equal feedings and fed at 09.00 and 17.00 hours. Fish were weighed once every 2 weeks and the daily ration adjusted accordingly. A photoperiod of 12 h light–12 h dark (light 08.00–20.00 hours) was used. The fish were fed the test diets for an 8-week period.

At the end of the feeding trial, the fish were weighed. Weight gain (as measured by the percentage of body-weight gain) and feed efficiency were calculated as described previously (Chou & Shiau, 1999). After the final weighing, four fish were randomly removed from each aquarium, gill samples were collected and pooled for Na^+–K^+ ATPase activity determination (Hwang *et al.* 1988). Four other fish were then taken randomly from each aquarium and pooled for body Na determination (Association of Official Analytical Chemists, 1995). In brief, 0·1 g homogenised fish was digested in a mixture of 5 ml nitric acid (65 % (v/v); Merck & Co., Darmstadt, Germany) and 2 ml H_2O_2 (30 % (v/v); Hayashi Pure Chemical Industries Ltd, Osaka, Japan) using a dry bath heater (Dry Bath Model 110001; Boekel, Feasterville, PA, USA). Body Na concentration was determined by flame atomic absorption spectroscopy (Z-5000; Hitachi Ltd, Tokyo, Japan).

Statistical analysis

Results were analysed by one-way ANOVA. When the ANOVA identified differences among groups, multiple comparisons among means were made with Duncan's new multiple-range test. Statistical significance was determined by setting the aggregate type I error at 5 % ($P<0·05$) for each set of comparisons. Dietary Na requirements for juvenile tilapia were estimated by the polynomial regression method (Zeitoun *et al.* 1976), the broken-line regression method (Robbins, 1986) and by linear regression analysis (Wilson & El Naggar, 1992).

Results

In the fresh-water-rearing environment, the weight gains were significantly ($P<0·05$) higher in the fish fed the diet supplemented with 2 g Na/kg diet than in the fish fed the diets supplemented with ≥ 3 and ≤0·5 g Na/kg diet

(Table 2). The pattern of feed efficiency was similar to that of weight gain. Survival of fish among the dietary groups was 93–100 %. Gill Na^+–K^+ ATPase activity was highest in the fish fed the diets supplemented with 1–3 g Na/kg diet, followed by the fish fed the diet with 7 g Na/kg diet and lowest in the fish fed the unsupplemented control diet. In the seawater-rearing environment, all these parameters were not significantly different among all the dietary groups (Table 3).

The weight gain of the fish reared in fresh water and their gill Na^+–K^+ ATPase activity *v.* dietary Na concentration were analysed by polynomial (cubic) regression (Zeitoun *et al.* 1976) and broken-line analysis (Robbins, 1986), respectively. As shown in Fig. 1, the Na requirement of tilapia is estimated to be 1·9 (weight gain) and 1·3 (gill Na^+–K^+ ATPase activity) g/kg diet.

Whole-body Na concentration in the fish reared in fresh water generally increased as the dietary Na supplementation level increased ($Y = 0{\cdot}03X + 0{\cdot}16$, r 0·98; Table 4), but this linear increase in body Na concentration was not observed in the fish reared in seawater (Table 5). When these data were used to calculate the whole-body Na retention for each group of fish, linear regression analysis of the dietary Na levels *v.* whole-body Na retention values (Fig. 2) indicated an Na requirement of 1·3 g/kg diet ($Y = -0{\cdot}70X + 0{\cdot}94$, r −0·99) for fish reared in fresh water, whereas no dietary Na is required for fish reared in seawater ($Y = -0{\cdot}58X + 0{\cdot}01$, r −0·99).

Discussion

The essentiality of dietary Na for normal growth of tilapia in fresh water is clearly demonstrated in the present study. Weight gain was reduced for the fish given the unsupplemented basal diet and increased with incremented increases in dietary Na up to the requirement level. Both food and the aquatic medium can be important sources of electrolytes for euryhaline fish. The Na content in the rearing water of the present study ranged from 8·51 to 15·34 mg/l in fresh water and from 10·62 to 12·42 g/l in seawater. For juvenile tilapia reared in fresh water, the addition of Na to the diet improved growth and feed efficiency in

Table 2. Weight gain, feed efficiency (FE), survival and gill Na^+–K^+ ATPase activity of fresh-water-reared tilapia (*Oreochromis niloticus* × *O. aureus*) fed diets containing various levels of sodium*

(Mean values and standard deviations)

Na supplementation level (g/kg)	Weight gain (%)		FE† (%)		Survival (%)		Gill Na^+–K^+ ATPase (μmol inorganic phosphate/mg protein per h)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	281[a]	11	0·61[a]	0·05	100	0	5·03[c]	0·09
0·5	294[a]	17	0·66[ab]	0·01	93	8·4	4·74[bc]	0·21
1	312[ab]	18	0·69[ab]	0·05	96	7·7	4·05[a]	0·31
2	330[b]	15	0·71[b]	0·01	96	3·9	4·01[a]	0·24
3	284[a]	8	0·66[ab]	0·04	100	0	4·13[a]	0·35
5	279[a]	14	0·62[a]	0·04	93	6·7	4·36[ab]	0·26
7	283[a]	13	0·62[a]	0·06	97	4·7	4·58[b]	0·10

[a,b,c] Mean values within a column, with unlike superscript letters are significantly different ($P < 0{\cdot}05$).
* Values are means of three groups of fish (initially fifteen fish per group) except for Na^+–K^+ ATPase activity determination (four fish per group).
† FE = g weight gain/g feed consumed.

Table 3. Weight gain, feed efficiency (FE), survival and gill Na^+–K^+ ATPase activity of seawater-reared tilapia (*Oreochromis niloticus* × *O. aureus*) fed diets containing various levels of sodium*

(Mean values and standard deviations)

Na supplementation level (g/kg)	Weight gain (%)		FE† (%)		Survival (%)		Gill Na^+–K^+ ATPase (μmol inorganic phosphate/mg protein per h)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	270	20	0·58	0·11	100	0·0	5·97	0·55
0·2	277	21	0·59	0·04	97	4·7	5·81	0·30
0·5	271	7	0·58	0·07	100	0·0	6·29	0·51
0·8	272	13	0·58	0·09	100	0·0	5·92	0·09
1·2	267	8	0·57	0·10	93	6·6	6·07	0·69
1·5	280	15	0·61	0·06	100	0·0	5·91	0·28
2	266	19	0·58	0·08	96	3·8	5·69	0·78
3	272	18	0·58	0·11	100	0·0	5·87	0·08

* Values are means of three groups of fish (initially fifteen fish per group) except for Na^+–K^+ ATPase activity determination (four fish per group).
† FE = g weight gain/g feed consumed.

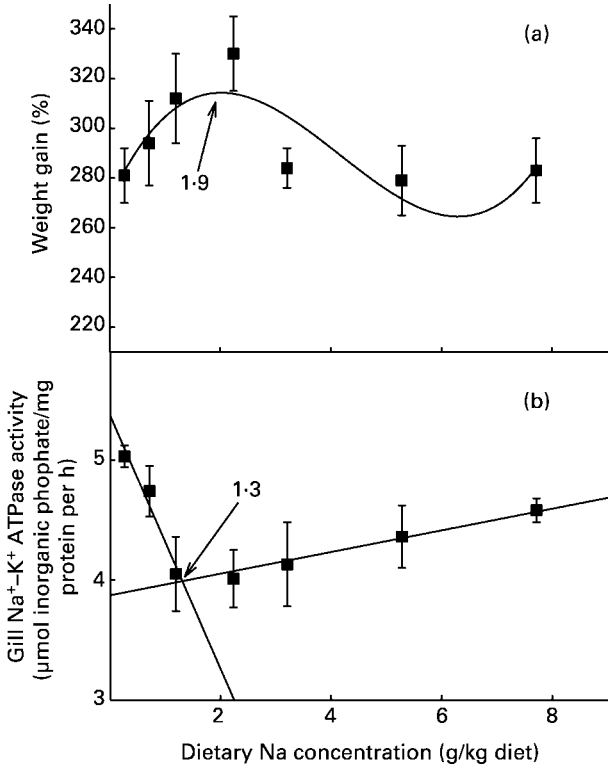

Fig. 1. The effect of dietary Na on relative weight gain (a) and gill Na^+–K^+ ATPase activity (b) of tilapia (*Oreochromis niloticus* × *O. aureus*) reared in fresh water. For details of diets, see Table 1 and for details of procedures, see p. 586. For weight gain, each point represents the mean of three groups of fish (initially fifteen fish per group); for Na^+–K^+ ATPase activity determination, each point represents the mean of three groups of fish (four fish per group). The vertical bars represent standard deviations. Requirements derived with the polynomial regression method for weight gain and with the broken-line regression method for gill Na^+–K^+ ATPase are 1·9 and 1·3 g/kg diet, respectively. For weight gain $Y = 1{\cdot}51X^3 - 17{\cdot}84X^2 + 52{\cdot}75X + 268{\cdot}74$, r 0·81. For gill $Na^+ - K^+$ ATPase activity $Y = -1{\cdot}06X + 5{\cdot}38$, r −0·97; $Y = 0{\cdot}10X + 3{\cdot}85$, r 0·96.

the present study. However, no such benefit was apparent for the fish in seawater.

Zeitoun *et al.* (1976) have suggested the use of polynomial regression analysis as a means of estimating the relationship between weight gain and essential nutrient intake. As indicated by Zeitoun *et al.* (1976), the value corresponding to maximal gain estimated by cubic regression is defined as the maximum concentration of dietary nutrient that produces optimal growth, and beyond which growth is depressed. In the present study, the weight gain of fish reached a maximum at 2 g Na supplementation/kg diet and decreased thereafter. Thus, the requirement was estimated by a polynomial regression analysis (Zeitoun *et al.* 1976).

Requirements for macronutrients such as protein, lipid, and essential amino acids of cultured fish are generally best defined in growing animals by growth data in feeding studies. Besides the growth parameters, attention has been drawn to the usefulness of tissue enzyme activity measurements as an adjunct to requirement studies in that they provide a functional measure of the nutritional status of a fish (Cowey, 1976). Gills are the most important extra-renal organs responsible for osmoregulation in fish. The biochemical mechanisms for the maintenance of constant levels of ions in body fluids depend on the activity of Na^+–K^+ ATPase, and the activities of gill Na^+–K^+ ATPase in euryhaline teleosts are affected by environmental salinities and ion concentrations (Hwang *et al.* 1989; Mayer-Gostan & Naon, 1992; McCormick, 1995; Shiau & Hsieh, 2001). The altered and unaffected gill Na^+–K^+ ATPase of tilapia reared in fresh water and seawater, respectively, found in the present study suggests that this variable may permit a satisfactory evaluation of Na status of the fish. The broken-line analysis of gill Na^+–K^+ ATPase activity in tilapia reared in fresh water (Fig. 1) suggests that gill Na^+–K^+ ATPase activity can be used to estimate the Na requirement of fish.

To the best of our knowledge, the present study is the first to establish the dietary Na requirement in fish. Several studies with euryhaline fish have indicated that dietary salt (NaCl) supplementation has negligible or even negative effects on growth and feed efficiency. Shaw *et al.* (1975) fed smolts of Atlantic salmon diets containing 4, 6 and 9 % NaCl in fresh water and 4, 6 and 12 % NaCl in seawater without observing any differences in fish growth or feed efficiency among treatments. MacLeod (1978) fed

Table 4. Whole-body sodium balance of fresh-water-reared tilapia (*Oreochromis niloticus* × *O. aureus*) fed diets containing various levels of sodium*

(Mean values and standard deviations)

Na supplementation level (g/kg diet)	Initial whole-body Na concentration (mg/g)	Total Na fed (mg/g)		Final whole-body Na concentration (mg/g)		Final−initial whole-body Na concentration (mg/g)		Whole-body Na retention† (mg/g)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	0·23	0·22	0·02	1·18	0·07	0·95	0·01	0·72	0·02
0·5	0·23	0·53	0·05	1·16	0·09	0·93	0·02	0·41	0·01
1	0·23	0·84	0·05	1·21	0·12	0·98	0·03	0·14	0·00
2	0·23	1·52	0·08	1·24	0·08	1·01	0·02	−0·51	0·02
3	0·23	2·51	0·12	1·29	0·13	1·06	0·03	−1·46	0·03
5	0·23	3·79	0·09	1·33	0·12	1·10	0·04	−2·69	0·03
7	0·23	5·67	1·80	1·43	0·11	1·20	0·04	−4·48	0·06

* Values are means of three groups of fish (four fish per group).
† Final−initial whole-body Na concentration−total Na fed.

Table 5. Whole-body sodium balance of seawater-reared tilapia (*Oreochromis niloticus* × *O. aureus*) fed diets containing various levels of sodium*

(Mean values and standard deviations)

Na supplementation level (g/kg diet)	Initial whole-body Na concentration (mg/g)	Total Na fed (mg/g)		Final whole-body Na concentration (mg/g)		Final−initial whole-body Na concentration (mg/g)		Whole-body Na retention† (mg/g)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	1·44	0·12	0·01	1·37	0·10	−0·07	0·00	−0·19	0·01
0·2	1·44	0·25	0·01	1·44	0·13	0·00	0·00	−0·25	0·02
0·5	1·44	0·42	0·01	1·46	0·15	0·02	0·00	−0·40	0·02
0·8	1·44	0·58	0·03	1·44	0·09	0·00	0·00	−0·58	0·03
1·2	1·44	0·80	0·02	1·42	0·16	−0·02	0·00	−0·82	0·02
1·5	1·44	0·95	0·03	1·45	0·12	0·01	0·00	−0·94	0·04
2	1·44	1·26	0·02	1·40	0·08	−0·04	0·00	−1·30	0·04
3	1·44	1·85	0·03	1·42	0·13	−0·02	0·00	−1·87	0·06

* Values are means of three groups of fish (four fish per group).
† Final−initial whole-body Na concentration−total Na fed.

practical diets supplemented with NaCl at 5 and 8·5 % to rainbow trout in fresh water and did not observe any effects on feed efficiency. In a similar study, Salman & Eddy (1988) noted that additions of NaCl at 4·5, 9·2, and 11·6 % to practical diets adversely affected growth and feed efficiency of rainbow trout in fresh water. In the present study, Na was incorporated into basal diets at 0·5, 1, 2, 3, 5, and 7 g/kg diet for tilapia reared in fresh water; these values are equivalent to 0·127, 0·254, 0·509, 0·763, 1·272, and 1·780 % of NaCl level, respectively. The optimal dietary Na supplementation level of 1·6 g/kg diet (equivalent to 0·406 % of NaCl) obtained in the present study may suggest that the NaCl supplementation levels used in these previous studies were too high.

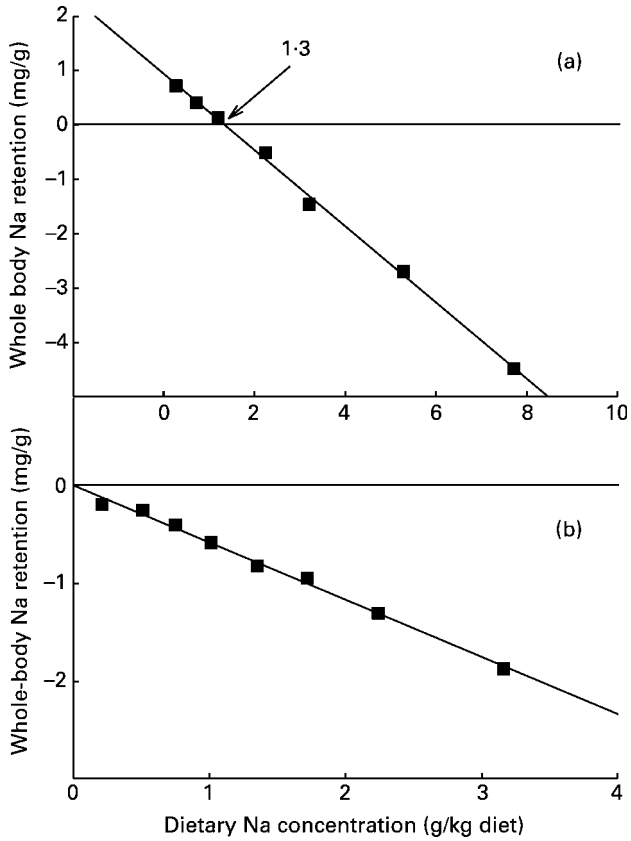

Fig. 2. The relationship between dietary Na concentration and whole-body Na retention in tilapia (*Oreochromis niloticus* × *O. aureus*) reared in fresh water (a) and seawater (b). For details of diets, see Table 1 and for details of procedures, see p. 586. Each point represents the mean of three groups of fish (four fish per group). The linear regression method suggests that 95 % of Y_{max} in this function is achieved with a dietary Na concentration of 1·3 g/kg diet, indicating the requirement for tilapia reared in fresh water (a), whereas no dietary Na is required for tilapia reared in seawater (b). For fresh-water rearing $Y = -0{\cdot}70X + 0{\cdot}94$, r −0·99; for seawater rearing $Y = -0{\cdot}58X + 0{\cdot}01$, r −0·99.

Murray & Andrews (1979) found no effects on growth for channel catfish (*Ictalurus punctatus*) in fresh water fed a basal diet (with 0·06 % Na) supplemented with NaCl at 0·25 to 2·0 %. Note, however, that Murray & Andrews' (1979) study was also not designed for the quantification of dietary Na requirement of channel catfish. Nevertheless, when the total Na content of the 0·25 % NaCl group in that study was calculated, a value of 1·583 g Na/kg diet (i.e. 0·983 g Na/kg of the supplementation plus 0·6 g Na/kg from the original basal diet) was obtained. This value is close to the optimal dietary Na requirement of tilapia (i.e. 1·6 g Na/kg diet) obtained in the present study. Not all fish respond well in growth rate to the dietary minerals. For example, the dietary K requirement of tilapia was quantified based on the weight gain of the fish (Shiau & Hsieh, 2001). However, a study with channel catfish showed that the weight gain of the fish did not respond to the dietary K supplementation (Wilson & El Naggar, 1992). The dietary K requirement of channel catfish was thus obtained by whole-body K retention. In the present study, as well as using the weight gain of the fish and their gill Na^+–K^+ ATPase activity to estimate the dietary Na requirement in tilapia, whole-body Na retention data were also calculated to estimate the requirement, and the values from the three variables agree well (Figs. 1 and 2).

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