Whole-body calcium flux rates in cichlid teleost fish *Oreochromis mossambicus* adapted to freshwater

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FLIK, G., J. C. FENWICK, Z. KOLAR, N. MAYER-GOSTAN, AND S. E. WENDELAAR BONGA. *Whole-body calcium flux rates in cichlid teleost fish Oreochromis mossambicus adapted to freshwater.* Am. J. Physiol. 249 (Regulatory Integrative Comp. Physiol. 18): R432–R437, 1985.—Radiotracer techniques were used to measure influx and efflux rates of Ca^{2+} in freshwater-adapted *Oreochromis mossambicus*. The influx rate of Ca^{2+} is related to body weight (W) as $F_{in} = 50W^{0.805}$ nmol Ca^{2+}/h. For a 20-g fish the calculated influx rate was 558 nmol Ca^{2+}/h, and this was attributed largely to extraintestinal uptake since the drinking rate was estimated to be only 28 μl water/h, which corresponds to an intake of 22.4 nmol Ca^{2+}/h. The Ca^{2+} efflux rate was calculated using the initial rate of appearance of radiotracer in the ambient water and the specific activity of plasma Ca^{2+}. Tracer efflux rates were constant over 6–8 h, which indicated that there was no substantial loss of tracer in either the urine or the feces because this would have resulted in random bursts of tracer loss. Efflux rates then primarily represent integumentary and presumably branchial efflux rates. The efflux rate of Ca^{2+} is related to body weight as $F_{out} = 30W^{0.563}$ nmol Ca^{2+}/h, which means an efflux rate of 162 nmol Ca^{2+}/h for a 20-g fish. The net whole-body Ca^{2+} influx, calculated as $F_{net} = F_{in} - F_{out}$, was 396 nmol/h for a 20-g fish, which proves that the ambient water is an important source of Ca^{2+}.

freshwater teleost; calcium influx rates; calcium efflux rates; growth; total body calcium pool

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FRESHWATER TELEOSTEAN FISH such as *Oreochromis mossambicus* (hereafter called tilapia) maintain their plasma Ca levels within narrow limits (5). As with most teleosts, this species continues to grow under natural conditions and must therefore continually increase its amounts of whole-body Ca. To satisfy this constant requirement for Ca^{2+} uptake and to compensate for Ca^{2+} losses caused by outward diffusion across the integument and via the production of urine and feces, freshwater fish actively accumulate Ca^{2+} from both food and water. However, direct absorption of Ca^{2+} from the water by the gills is believed to be the predominant route for Ca^{2+} uptake in freshwater fish (1, 2, 13). This report deals with the significance of the extraintestinal Ca^{2+} uptake.

The understanding of Ca^{2+} fluxes in teleost fish is limited by a paucity of studies. Furthermore, most studies concerned with Ca^{2+} handling by fish involved seawater species only. Seawater contains high levels of Ca^{2+}, and in such an environment fish may be forced to compensate for excessive influx of Ca^{2+} by secreting Ca^{2+} or reducing Ca^{2+} influx. Conversely, active uptake of Ca^{2+} is a requisite for survival and growth in freshwater, where Ca^{2+} concentrations are often much lower than those of the body fluids. Moreover, the osmolarity and the concentrations of ions other than Ca^{2+} are very much different in freshwater than in seawater. Ambient concentrations of one particular ion may well affect the fish's physiological activity with respect to another ion. The high levels of Mg^{2+} in seawater, for example, influence the Ca^{2+} physiology of fish adapted to Ca^{2+}-deficient seawater (28).

This study forms part of our investigation on the effect of environmental conditions and the endocrine control of Ca^{2+} metabolism in tilapia and deals with whole-body influx and efflux rates of Ca^{2+} in tilapia adapted to artificial freshwater containing 0.8 mM $CaCl_2$. Although Ca^{2+} influx data are available for a limited number of freshwater species (1, 2, 6, 7, 12, 14, 17, 23), to our knowledge this is the first report on both directly determined influx rates and efflux rates of Ca^{2+} for freshwater fish. Such directly determined efflux rates are essential to assess net uptake of Ca^{2+} from the water and to evaluate the role of ambient water as a Ca^{2+} source for fish. These data enabled us to estimate the contribution of Ca^{2+} taken up from the water to the Ca balance of the fish.

MATERIALS AND METHODS

Male tilapia, *Oreochromis mossambicus* (formerly called *Sarotherodon mossambicus*), were obtained from laboratory stock and kept in Nijmegen tap water. The conditions were the same as those described earlier (30), but the temperature was 28 ± 1°C.

Fish used for radiotracer studies were transferred into 100-liter aquariums containing artificial freshwater made up from demineralized water and containing (in mM) 3.8 NaCl, 0.06 KCl, 0.2 $MgSO_4$, and 0.8 $CaCl_2$. The pH was adjusted with $NaHCO_3$ to 7.4 ± 0.2, and the osmolarity was 8–10 mosmol/l.

Although the composition of the artificial freshwater is essentially the same as the composition of the Nijme-

gen tap water, artificial freshwater was preferred for radiotracer studies to guarantee constant concentrations of Ca^{2+} and other ions. During the experiments the pH and Ca^{2+} concentration in water was monitored and adjusted if necessary. Nitrogen wastes were kept in all cases below 2 μM NH_4^+.

Calcium determination. Total body Ca was determined by digesting fish in concentrated HNO_3. Blood was collected from the caudal blood vessels, and plasma samples were prepared as previously described (30). Total Ca of water, HNO_3^- digests, and plasma was analyzed by means of atomic absorption spectrophotometry, using $LaCl_3$ (20 mM) as diluent. To avoid contamination of the atomic absorption unit, $^{45}Ca^{2+}$-containing samples were determined with a commercial Ca kit (Sigma).

Radiotracer techniques. To follow the Ca^{2+} transport, radiotracers $^{45}Ca^{2+}$ and $^{47}Ca^{2+}$ were used. Both were purchased (Amersham International, England) as $CaCl_2$ in aqueous solution. Specific activities were 9.25–37.5 GBq/mol Ca and >0.74 GBq/mol Ca for ^{45}Ca and ^{47}Ca, respectively. ^{45}Ca decays (half-life 164 days) by β^- emission (E_{max} 0.252 MeV) into stable ^{45}Sc. Its activity was measured by liquid-scintillation counting (LKB Rackbeta LSA, equipped with a dpm program) of samples prepared by mixing 1 ml of a $^{45}Ca^{2+}$-containing aqueous solution with 4 ml counting solution (Aqualuma, Lumac). $^{47}Ca^{2+}$ decays (half-life 4.54 days) by β^- emission followed by the emission of gamma rays [E = 0.49 (5%), 0.815 (5%), and 1.308 (74%) MeV] into ^{47}Sc, which also decays (half-life 3.40 days) by β^- emission followed by the emission of gamma rays [E = 0.160 (73%) MeV]. The $^{47}Ca^{2+}$ activity in liquid samples and in whole fish was measured by a well-type 3 × 3 in. NaI (Tl) scintillation detector equipped with an appropriate counter (gamma ray spectrometer: Elscint or LKB Ultrogamma II) set to measure 1.308 MeV photopeak of $^{47}Ca^{2+}$ only. In this setting the contribution of ^{47}Sc emission was completely excluded.

The counting efficiency for the 1.308-MeV radiation of a 1-ml sample measured in the well amounted to ~15% (count·s^{-1}·0.74 Bq^{-1} × 100). A linear decrease of the counting rate (4 and 6.5% decrease per ml for the aforementioned detectors, respectively) was observed with increasing sample volume. As a routine for all but the 1-ml samples, appropriate corrections were made, which led to an apparent counting rate corresponding to 1-ml sample volume.

Whole-body $^{47}Ca^{2+}$ activity of live fish was measured using a Perspex container with a ring-shaped compartment (1 liter) mounted concentrically around a well-type scintillation crystal. To ascertain that differences in whole-body counts determined for individual fish did not derive from differences in position of the fish in the ring-shaped compartment, 10 fish were subjected to the counting procedure 10 times in a row; average standard deviation in whole-body counts was 2.35 ± 0.74% (n = 10). The following procedure was applied to determine the counting efficiency for 1.308 MeV gamma rays for $^{47}Ca^{2+}$ in the fish when measured alive relative to the counting efficiency for samples measured in the well of the scintillation crystals. A $^{47}Ca^{2+}$-containing fish was rinsed (1 min) with tracer-free water to remove adsorbed tracer, and the fish was counted in the Perspex container. Then the fish was killed and quickly dissolved in 12 M KOH at 60°C, and the bones were destroyed mechanically. The resulting digest was divided into 5-ml portions that were counted in the well of the scintillation crystal. From the sum of the counting rates of all 5-ml portions and the counting rate obtained in the Perspex container for the whole live fish, the relative counting efficiency was calculated (fish counting rate/counting rate in well × 100). For fish of 10–30 g, values of 19.0 ± 1.3% (n = 5) were obtained. The absolute counting efficiency for intact fish came to 2.7% in this set up (count·s^{-1}·0.74 Bq^{-1} × 100).

Animals were always counted after a 1-min rinse in tracer-free water to remove tracer adsorbed to the body surface. After being counted, the fish were removed from the container; then water was counted for background activity. Counts collected for live fish surpassed at least 10 times background count rates. During transfers fish were handled with wet rubber gloves to prevent any skin damage that could influence the ion fluxes (12). Amounts of fish during the influx studies and tracer retention studies never exceeded 15 g fish/l H_2O.

Calcium influx. For Ca^{2+} influx studies all-glass aquariums or Perspex containers contained 12 or 1 liter of well-aerated water, respectively. At the start of influx experiments (2–8 h after addition of tracer to water), fish were transferred to the exposure systems; they were not fed during influx experiments that lasted up to 3 h.

Drinking rates. Drinking rates were determined on the basis of gut $^{47}Ca^{2+}$ contents as reported by Pang et al. (23). Immediately after 3 h exposure to tracer, the abdominal cavity was opened, and the intestinal tract, minus liver and gall bladder, was assessed for $^{47}Ca^{2+}$ radioactivity. Good care was taken to include the total intestinal contents. The volume of water consumed was calculated (gut count·s^{-1}/count·s^{-1}·ml $water^{-1}$; drinking rates were expressed as μl water/h or nmol Ca^{2+}/h).

Calcium efflux. First, we studied the retention of intraperitoneally injected $^{47}Ca^{2+}$ (4.44 × 10^7 Bq/g). Fish were kept in all-glass aquariums with 40 liters water; the water was circulated through charcoal filters that were previously equilibrated to the water. Filters and solutions were renewed if necessary to keep tracer activity at background levels to prevent tracer backflux. During tracer-retention experiments fish were normally fed. Second, fish were given a dose of $^{45}Ca^{2+}$ (D^f), and the plasma radioactivity was measured from 0.5 to 79 h after injection. Plasma radioactivity (q_p) was expressed as (q_p/V_p)D^f, where V_p is plasma volume. To determine Ca^{2+} efflux rates fish were injected intraperitoneally with $^{45}Ca^{2+}$ (2.88 × 10^8 Bq/g) and were held and fed for 3 days. Then feeding was discontinued, and fish were starved for 1 day. Next, they were caught, the urinary bladder emptied by gently pressing the posterior abdominal wall, and the fish transferred to Perspex containers with 0.5–1 liters aerated water for 6–8 h. During this period and at the end of the experiment water samples were taken and their radioactivity (q_w) determined. At the completion of some experiments an aliquot of concentrated EGTA solution was added to the water to obtain a concentration of ~5 mM, and q_w was established.

No significant differences were observed for q_w determined in the presence or absence of EGTA, indicating that no significant adsorption of Ca^{2+} tracer to the container walls occurred.

Calculations. The influx rates of Ca^{2+} (F_{in}) were calculated from the time curves for the tracer content of the fish (q_f) normalized to the tracer content of the water at zero time (q_{w0}). The instantaneous initial upslope of these curves at zero time, $d(q_f/q_{w0})/dt$, is equal to F_{in}/Q_w, where Q_w is amount of Ca in the water (26). Because of the inaccuracies in defining the early portion of the curve via observed points, a slope obtained by the least-squares fitting of a line through the data points for up to 3 h was used instead of the actual slope at zero time.

The efflux rates of Ca^{2+} (F_{out}) were calculated from the time curves for the tracer content of the water (q_w), which one may normalize to the tracer content of the blood plasma of the fish at zero time (q_{p0}). Zero time is the moment of the immersion into tracer-free water of fish previously injected with tracer and kept for 3 or 4 days in tracer-free water. The slope of these curves at zero time, $d(q_w/q_{p0})dt$, is equal to F_{out}/Q_p, where Q_p is amount of Ca in the plasma of the fish. The ratio q_{p0}/Q_p is equal to the specific activity of Ca^{2+} in the plasma at zero time (SA_{p0}); hence $F_{out} = (dq_w/dt)/SA_{p0}$. Since SA_{p0} is not accessible for direct determination, SA_p at the end of the experiments was used instead (SA_p was shown not to decrease significantly over a 6- to 8-h period 4 days after tracer injection). Here also the actual slopes at zero time were not used, but the slopes obtained by least-squares fitting of a line through the data points for up to 8 h.

Statistics and notations. Student's t test for unpaired observations or the Kruskal-Wallis one-way ANOVA by ranks was applied to assess statistical significance of differences of mean values. Linear regression analysis was based on the least-squares method. The symbols, definitions, and units used were taken from Brownell et al. (3) and Shipley and Clark (26):

q_a	quantity of tracer in compartment a (count/min, disintegration/min)
Q_a	quantity of tracee (material traced) in compartment a (mol)
V_a	medium volume of compartment a (liters)
SA_a	specific activity in compartment a; $SA_a = q_a/Q_a$
F	flux rate (mol/h)
D^a	dose of tracer administered to compartment a
W	wet wt (g)

Subscripts

f	fish
w	water
p	plasma
0	zero time

RESULTS

In freshwater male tilapia ranging in body weight from 4 to 93 g, the Ca content of the body was 316.3 ± 2.3 μmol/g. A full logarithmic plot of the results (Fig. 1) yields the power function for the total fish Ca^{2+} pool: $Q_f = 357.5W^{0.965}$ μmol Ca. The calculated slope of the regression line (0.965) approximates unity, which means that the size of the total body Ca pool is directly related to body weight.

Calcium influx and drinking rates. Intact fish accumulated $^{47}Ca^{2+}$ at a constant rate for at least 3 h (Fig. 2). Drinking rates determined on the basis of intestinal $^{47}Ca^{2+}$ contents were 28.0 ± 14.2 μl/h for a 20-g fish, i.e., an intake of 22.4 ± 12.2 nmol Ca^{2+}/h. The radioactivity present in the gut after 3 h as a fraction of the estimated total body radioactivity averaged 1.26% and never exceeded 2.6%. Thus although the slope of the tracer uptake curve reflects both the entry through the integument and the gut contents (the latter belong to external compartment), whole-body Ca^{2+} influx can be calculated from the slope of a 3-h tracer uptake curve and the specific activity of $^{47}Ca^{2+}$ in the water. Since the fish size varied from 9.8 to 28 g in this group, we could establish a relation between influx rates and body weight. A positive correlation was observed between body weight and Ca^{2+} influx. Rates of Ca^{2+} influx ranged from 195 nmol/h (W = 9.8 g) to 1,065 nmol/h (W = 28 g) (Fig. 3). In this weight range Ca^{2+} influx rate is related to body weight as $F_{in} = 50W^{0.805}$ nmol/h. For a 20-g fish F_{in} comes to 558 nmol/h Ca^{2+}.

To assess whether Ca^{2+} influx in our fish underwent

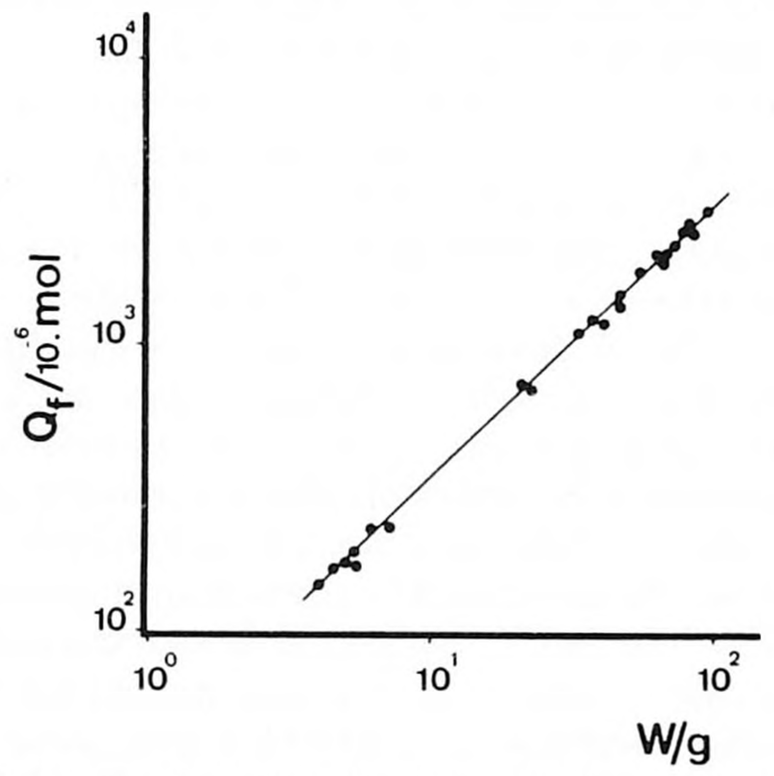

FIG. 1. Full logarithmic plot of relationship between body weight in g (W/g) and whole-body Ca content in μmol ($Q_f/10^{-6}$ mol). A highly significant correlation was observed between log(W/g) and log($Q_f/10^{-6}$ mol) ($r_0 = 0.998$, $P < 0.001$, $n = 25$). Total body Ca for freshwater male tilapia can be described by $Q_f = 357.5W^{0.965}$ μmol Ca.

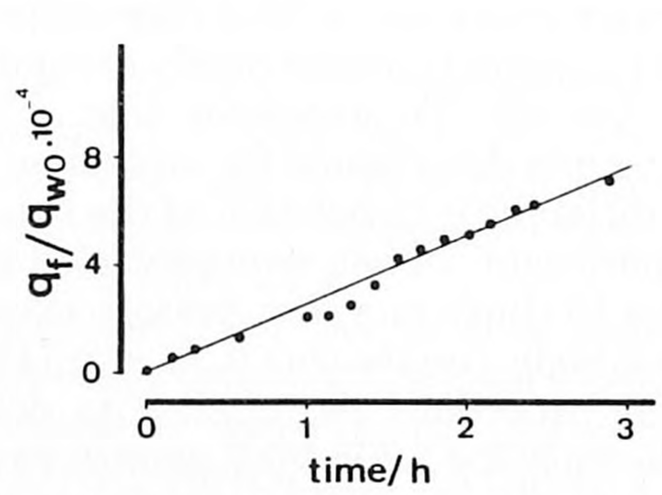

FIG. 2. Accumulation of $^{47}Ca^{2+}$ (q_f/q_{w0}) in an individual fish expressed as a fraction of totai radioactivity in water at zero time. Ca^{2+} influx rate is calculated from slopes of such linear tracer uptake curves and total water Ca^{2+} pool. Calculated Ca^{2+} influx rate for this fish (W = 15.1 g) was 880 nmol/h.

fluctuation throughout the year, all influx rates of individual fish [$F_{in}(W)$] presented in Fig. 3 were converted to values for a 20-g fish according to $F_{in}(20) = F_{in}(W) \cdot (20/W)^{0.805}$. These values were subsequently pooled in groups per month of experimentation. These data (Fig. 4) showed that Ca^{2+} influx rates in our freshwater male tilapia did not undergo statistically significant ($P > 0.20$) seasonal fluctuation.

Calcium efflux rates. For determination of efflux rates plasma Ca^{2+} tracer specific activity should preferably be constant during the experiment. Figure 5 shows that in live tilapia the first 24 h after tracer injection were marked by a rapid decrease in whole-body tracer content. This was followed by a small, apparently linear decrease that lasted at least a further 60 h. In a separate experiment, using $^{45}Ca^{2+}$, plasma tracer activity ran concurrently with whole-body tracer content 24–79 h after tracer injection. Plasma tracer contents [$(q_p/V_p)D^f$] at 72 and 79 h, time of efflux experiments, were $(1.079 \pm 0.114) \times 10^{-3}$ ml^{-1} ($n = 4$) and $(1.058 \pm 0.327) \times 10^{-3}$ ml^{-1} ($n = 10$), respectively; these values did not differ in a statistically significant way ($P > 0.15$). From these observations we conclude that between 72 and 100 h after injection of tracer, its loss from the body is slow and linear and that the plasma Ca^{2+} tracer specific activity will not change significantly over a 6- to 8-h period. For 10 fish killed 79 h after tracer injection, plasma total Ca^{2+} amounted to 2.85 ± 0.13 mM; such values are in line with previously reported plasma Ca^{2+} levels in this species (30).

Tracer accumulation rate in the water proved to be constant over a 6- to 8-h period (Fig. 6). On the basis of the data presented in Fig. 4 and the accumulation rates of tracer in the water, Ca^{2+} efflux rates (Fig. 3) were calculated. In the body weight trajectory studied, efflux rates of Ca^{2+} were related to body weight as $F_{out} = 30W^{0.563}$ nmol/h. For a 20-g fish F_{out} was 162 nmol Ca^{2+}/h. According to the conservation equation $F_{net} = F_{in} - F_{out}$, a net influx rate of 392 nmol Ca^{2+}/h was calculated for a 20-g male tilapia in freshwater.

For a group of seven fish a growth rate of 0.15% body wt/day was measured over 61 days. By applying the relationship for total Ca^{2+} and body weight, $Q_f = 357.5 W^{0.965}$ μmol Ca, a mean body accumulation rate of 383 nmol Ca^{2+}/h was calculated from the observed growth rates.

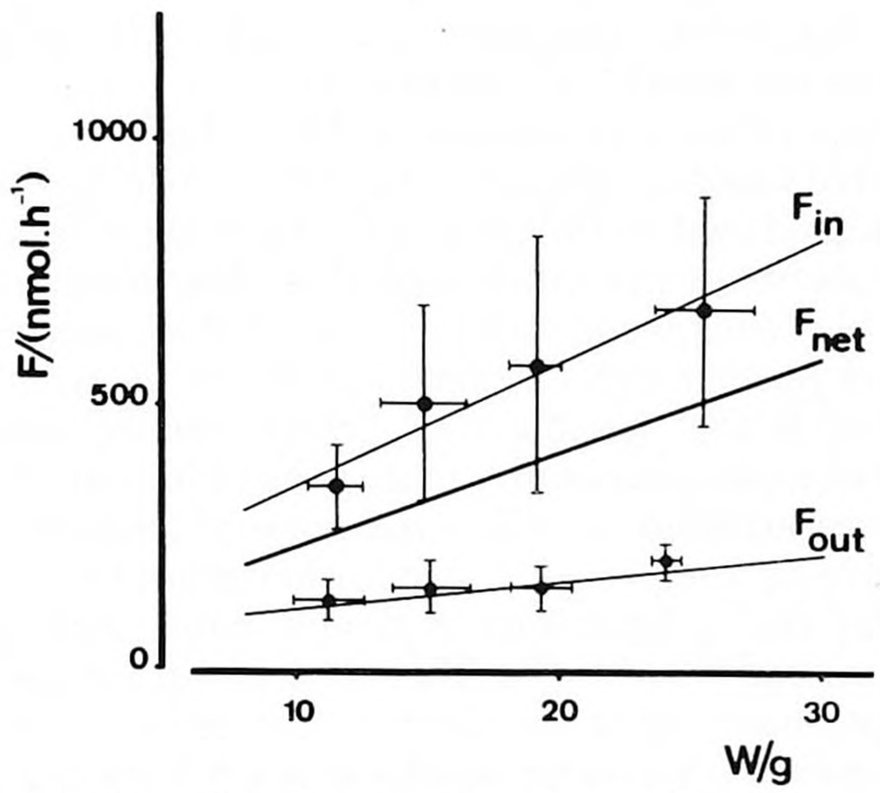

FIG. 3. Relationships between whole-body Ca^{2+} flux rates and body weight. For fish in range of 9–28 g, a significant positive correlation ($r_0 = 0.540$, $P < 0.001$, $n = 58$) was observed between Ca^{2+} influx rate (F_{in}) and body weight. Ca^{2+} influx rates were satisfactorily described by $F_{in} = 50W^{0.805}$ nmol/h. For fish in range of 9.8–24 g, Ca^{2+} efflux rates were positively correlated with body weight ($r_0 = 0.443$, $P < 0.05$, $n = 21$). Ca^{2+} efflux rates were satisfactorily described by $F_{out} = 30W^{0.563}$ nmol/h. *Thick line*, net Ca^{2+} influx rates calculated as difference between F_{in} and F_{out}.

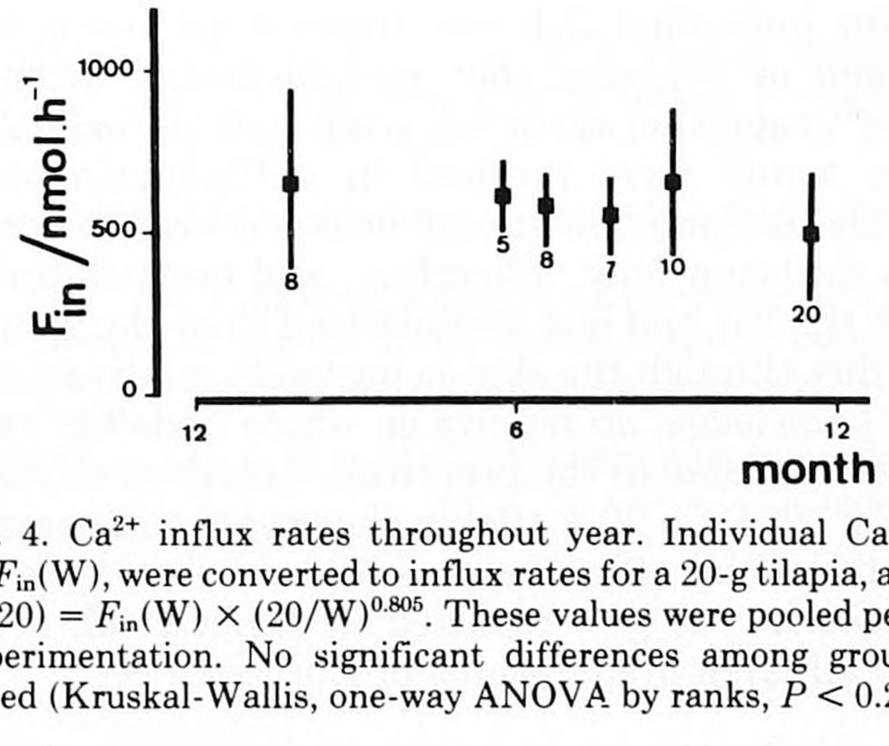

FIG. 4. Ca^{2+} influx rates throughout year. Individual Ca^{2+} influx rates, $F_{in}(W)$, were converted to influx rates for a 20-g tilapia, according to $F_{in}(20) = F_{in}(W) \times (20/W)^{0.805}$. These values were pooled per month of experimentation. No significant differences among groups were observed (Kruskal-Wallis, one-way ANOVA by ranks, $P < 0.20$).

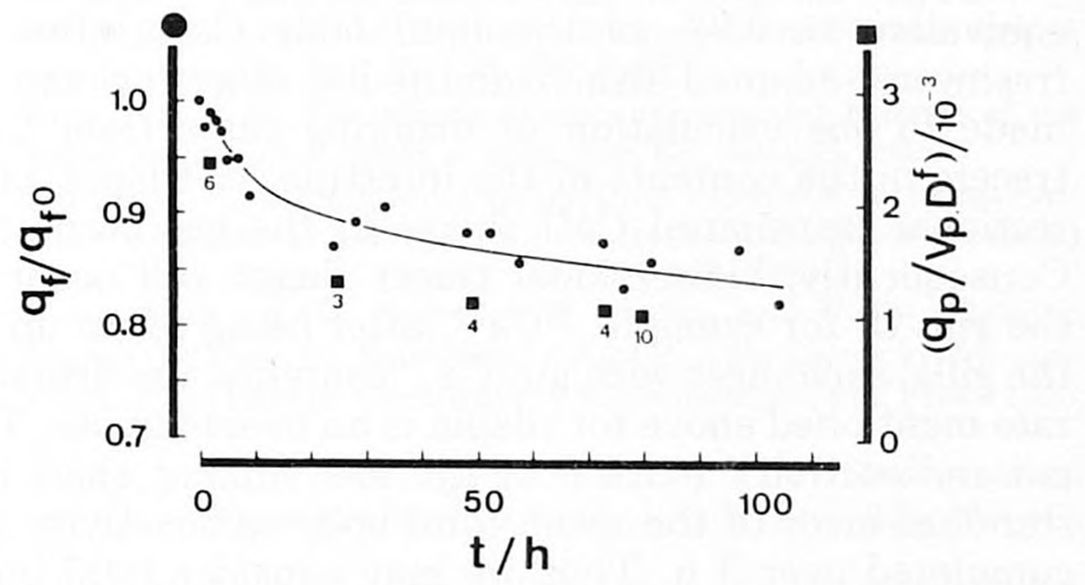

FIG. 5. Whole-body tracer retention curve for tilapia injected intraperitoneally with $^{47}Ca^{2+}$. Tracer retained in body (q_f) is expressed as a fraction of tracer in fish at zero (injection) time (q_f/q_{f0}). ●—● Mean values of 7 fish. Plasma tracer content (q_p/V_p) expressed as a fraction of dose of tracer injected (D^f) decreases concurrently with whole-body tracer content (■). Numbers indicate number of fish per group.

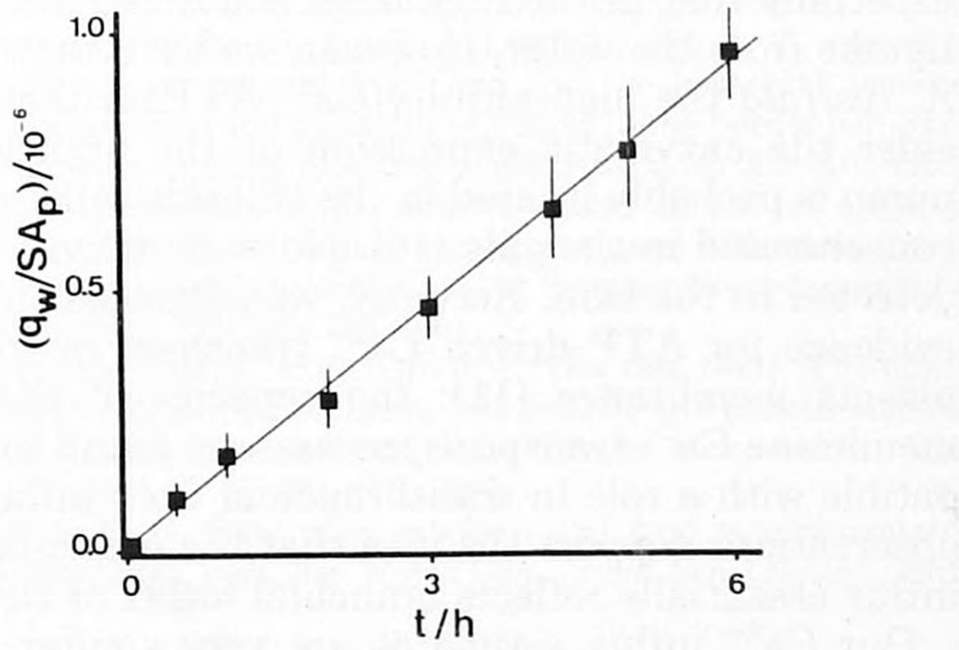

FIG. 6. Tracer appearance in water on immersion of $^{45}Ca^{2+}$-injected tilapia in tracer-free water. Water tracer content (q_w) is expressed as fraction of plasma $^{45}Ca^{2+}$ specific activity of fish at end of experiment (SA_p). Means ± SE for 10 fish are given ($r_0 = 0.999$, $P < 0.001$).

DISCUSSION

Calcium influx. To estimate Ca^{2+} uptake from the water in tilapia we determined bidirectional Ca^{2+} fluxes between fish and water. An influx rate of 558 nmol Ca^{2+}/h for a 20-g tilapia (27.9 nmol·h^{-1}·g^{-1}) was calculated, which is of the same order as influx rates reported for other species, e.g., *Fundulus kansae* (27 nmol·h^{-1}·g wet wt^{-1}; 7), *Carassius auratus* (16.3 nmol·h^{-1}·g $fish^{-1}$; 1), and *F. heteroclitus* (32.5 nmol·h^{-1}·g $fish^{-1}$; 23), but significantly lower than the Ca^{2+} influx rate for *C. auratus* (149 nmol·h^{-1}·g $fish^{-1}$) reported by Ichii and Mugiya (14).

Ca^{2+} from the water enters the fish via the gut by drinking water and via the integument, of which the gills form a major part (13). The drinking rate of 28 μl water/h for a 20-g fish found in this study is about half that of the values reported by Potts et al. (25) for the same species in freshwater (0.26% body wt/h), but we do not consider this difference physiologically significant. Reported values for other species follow (in μl·h^{-1}·100 g^{-1}): *C. auratus*, 51 (19); *Anguilla anguilla*, 135 (15); freshwater *Platichthys flesus*, 37 (15); *Salmo trutta*, 45.5 (21). If we assume that all Ca^{2+} entering tilapia by drinking is absorbed from the gut, the intestinal influx would amount to 22.4 nmol Ca^{2+}/h for a 20-g fish, which is equivalent to 3.9% of the total body Ca^{2+} influx in freshwater-adapted fish. Admittedly, objection can be made to the calculation of drinking rates from Ca^{2+} tracers in the contents of the intestine. In tilapia, bidirectional transmural Ca^{2+} fluxes in the gut occur (8). Consequently, bidirectional tracer fluxes will occur in the gut. If, for example, $^{47}Ca^{2+}$, after being taken up by the gills, exchanges with gut Ca^{2+} contents, the drinking rate mentioned above for tilapia is an overestimate. The gut radioactivity (<2.6% of q_f) was smaller than the standard error of the mean total body radioactivity accumulated over 3 h. Thus we may consider total body radioactivity accumulated over a 3-h period to approximate the extraintestinal influx of $^{47}Ca^{2+}$ for at least 97.4% or more. The gills, which represent 70–80% of the total body surface (13, 20), are considered the main site for Ca^{2+} entry in fish (24), although Mashiko and Jozuka (16) suggested that in freshwater *C. auratus*, the skin, especially that of the fins, is an additional site for Ca^{2+} uptake from the water. However, we have shown that in *A. rostrata* the high-affinity Ca^{2+}-ATPase that we consider the enzymatic expression of the branchial Ca^{2+} pump is probably located in the chloride cells, which are concentrated in the gills (10). No such activity could be detected in the skin. Recently, we advanced substantial evidence for ATP-driven Ca^{2+} transport in tilapia gill plasma membranes (11); the capacity of the plasma membrane Ca^{2+} transport process was found to be compatible with a role in transbranchial Ca^{2+} influx. These observations support the idea that the whole-body Ca^{2+} influx essentially reflects branchial influx of Ca^{2+}.

Our Ca^{2+} influx estimates are very similar to those reported for isolated head preparations of freshwater trout (24) but not to those reported on the gill arch preparation of European eel by Milet et al. (18). The observed relationship between body weight and Ca^{2+} influx rate ($F_{in} = 50W^{0.805}$ nmol/h) is strikingly similar to the relationship between body weight and water exchange reported for freshwater tilapia by Potts et al. (25). A similar function with a power of 0.85 was reported for the relationship between body weight and oxygen consumption in fish (31). The spread in individual values for whole-body Ca^{2+} influx rate may result from individual differences in general metabolic activity or Ca^{2+} status of the fish. Alternatively one might assume the existence of a direct relationship between gill surface and Ca^{2+} influx rates. Because the gill surface area is reported to vary considerably for individuals of the same species (13), this could explain the observed individual variation.

In our study we observed no seasonal variation in Ca^{2+} influx rates. That fish were kept under constant conditions throughout the year and only males were used may, however, have eliminated any season- or sex-related variations. Fleming et al. (7) reported seasonal variations in Ca^{2+} influx in *F. kansae*, which they tentatively related to changes in the endocrine status of the fish. Seasonal variations in responses to epinephrine have been reported for *Salmo gairdneri* (22), and such variations influence branchial Ca^{2+} uptake (24).

Calcium efflux. Calculations of whole-body Ca^{2+} efflux rates were based on plasma total $^{45}Ca^{2+}$ specific activity. We assumed that no differences in tracer specific activity existed between protein-bound Ca^{2+} fractions and the dialyzable (complexed and ionic) Ca^{2+} fractions, neither in blood plasma nor extracellular fluids. A second assumption is that plasma Ca^{2+} tracer specific activities did not change significantly during the efflux experiment. This was justified by the constancy of plasma tracer levels during the period of experimentation (i.e., on 4th day after tracer injection). A double constraint applies to the calculation of Ca^{2+} efflux rates in our set up. First, the experiment must be short in comparison with the time required for isotope equilibration between fish and water. Second, during the experiment the specific activity in the compartment from which the tracer leaves should be constant. Apparently both conditions were fulfilled, as was indicated by the constancy of efflux rate values during the experimental period (Fig. 6).

The question then arises to what extent whole-body efflux rates of Ca^{2+} reflect branchial efflux rates. In addition to branchial Ca^{2+} efflux, urinary and intestinal excretion of Ca^{2+} occur. The tracer accumulation in the water for individual fish was linear over 6–8 h, which was taken as evidence that no substantial urinary or fecal Ca^{2+} excretion occurred, since such periodical phenomena would have resulted in a fluctuating efflux. Efflux via the integument may be considered to occur by passive diffusion only. Therefore, and because the skin is much thicker and less vascularized than the gills (29), Ca^{2+} efflux through the skin is probably relatively small. To our knowledge, no reports on whole-body Ca^{2+} efflux rates are available in the literature. An efflux rate of 229 nmol Ca^{2+}/h in a 20-g tilapia, however, compares well with a branchial efflux rate of 6.93 μmol·h^{-1}·kg $fish^{-1}$ (139 nmol·h^{-1}·20 g^{-1}) reported by Milet et al. (18) for isolated gill arch preparations of European eel.

Net calcium flux. A freshwater-adapted tilapia of 20 g has a net uptake rate of Ca^{2+} from the water of ~400 nmol/h. This uptake rate will allow a freshwater tilapia of 20 g to grow by 1 g, equivalent to the accumulation of 310 μmol Ca^{2+} in the body, in 33 days. This value is commensurate with growth rate under our laboratory conditions. An experiment whereby tilapia were fed a Ca^{2+}-deficient diet did not significantly affect their growth rate (unpublished results). Apparently, tilapia have an efficient system to extract Ca^{2+} from the water. Berg (1, 2) has shown for *C. auratus* that intestinal Ca^{2+} absorption and branchial Ca^{2+} influx are inversely related. We have preliminary evidence that in tilapia the Ca^{2+} uptake from the water can be hormonally stimulated (27). It would be interesting to investigate whether the process of adjusting branchial Ca^{2+} uptake to the dietary supply in tilapia is mediated by hormones.

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