

EFFECT OF LOW AMBIENT MINERAL CONCENTRATIONS ON THE ACCUMULATION OF CALCIUM, MAGNESIUM AND PHOSPHORUS BY EARLY LIFE STAGES OF THE AIR-BREATHING ARMoured CATFISH *MEGALECHIS PERSONATA* (SILURIFORMES: CALLICHTHYIDAE)

JAN H. MOL^{1,*}, WIM AT SMA², GERT FLIK², HANS BOUWMEESTER³ AND JAN W. M. OSSE³

¹University of Suriname, CELOS, PO Box 9212, Paramaribo, Surinam, ²Department of Animal Physiology, University of Nijmegen, Toernooiveld 25, 6525 ED Nijmegen, The Netherlands and ³Department of Experimental Animal Morphology and Cell Biology, Wageningen Agricultural University, PO Box 338, 6700 AH Wageningen, The Netherlands

*e-mail: nzcs@cq-link.sr

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Summary

The accumulation of calcium, magnesium and phosphorus was measured during an 8-week period in the early life stages of the air-breathing armoured catfish *Megalechis personata* acclimated to low-mineral fresh water (0.073 mmol l⁻¹ calcium, 0.015 mmol l⁻¹ magnesium, <0.001 mmol l⁻¹ phosphate) and high-mineral fresh water (0.59 mmol l⁻¹ calcium, 1.94 mmol l⁻¹ magnesium, <0.001 mmol l⁻¹ phosphate). The fish accumulated calcium twice as fast and phosphorus 1.5 times as fast in low-mineral fresh water (LMF) as in high-mineral fresh water (HMF), while the rate of accumulation of magnesium did not differ in LMF and HMF. The difference in the rates of accumulation of calcium and phosphorus between LMF and HMF was independent of the growth performance (food intake) in LMF and HMF. The mineral content of young *M. personata* from natural swamps and rainforest creeks in Suriname followed the LMF accumulation curves. The transition from aquatic respiration to bimodal

respiration in the third week after hatching did not affect rates of mineral accumulation. The high rates of accumulation of calcium and magnesium of *M. personata* in LMF of 654 and 58 µmol h⁻¹ kg⁻¹, respectively, exceed the rates of uptake of calcium and magnesium of teleosts reported in the literature. The high rates of mineral accumulation in the early life stages of *M. personata* reflect the exponential growth during the first 8 weeks after hatching and the requirements of the juveniles while building their dermal armour. *M. personata* is well-adapted to neotropical fresh waters with an extremely low mineral content. The accumulation of calcium and phosphorus is discussed in relation to the function of the bony armour of *M. personata*.

Key words: mineral accumulation, low ambient mineral level, calcium, magnesium, phosphorus, armoured catfish, *Megalechis personata*.

Introduction

Freshwater fish accumulate minerals for growth either directly through uptake from the water by absorption of cations (inorganic phosphate is taken up *via* intestine only) across the gills or from the food across the intestinal wall (Berg, 1970; Maetz, 1974; Simkiss, 1974; Flik et al., 1993; Potts, 1994). In the gills of adult fish, chloride cells, or ionocytes, harbour the enzymatic machinery that drives the uptake of ions (Jürss and Bastrop, 1995). In fish larvae, ionocytes may also occur throughout the skin (Hwang, 1989) as long as the gills are underdeveloped. In freshwater fish, blood plasma concentrations of free Ca²⁺ range from 1 to 3 mmol l⁻¹ and of Mg²⁺ range from 0.3 to 2.1 mmol l⁻¹ (Mangum et al., 1978; Hanssen et al., 1989, 1992; van der Velden et al., 1989). Low ambient mineral concentrations and high H⁺ concentrations pose problems for the regulation of the mineral balance in

developing and adult fish (Nelson, 1982; Flik et al., 1986; Gonzalez and Dunson, 1987; van der Velden et al., 1991a; Gonzalez, 1996). Protons compete for ion-binding sites on specific carriers (Potts, 1994).

Neotropical rainforest creeks and black- and clear-water rivers (terminology of Sioli, 1950) are characterized by extremely low concentrations of dissolved inorganic ions. Concentrations of Ca²⁺ and Mg²⁺ may be as low as 0.9±0.8 and 1.5±0.6 µmol l⁻¹, respectively, and the conductance (at 25 °C) is usually below 20 µS cm⁻¹ (Furch, 1984). Moreover, black-water rivers are naturally acidic (pH 3–5) as a result of the presence of organic acids originating from decaying vegetation. The water chemistry clearly affects the biota of neotropical streams (Junk and Furch, 1985). In two species of discus fish, the mineral composition of the vertebrae reflected

a difference in the mineral content of the water in which they lived (Geisler and Schneider, 1976): in white water with relatively high ion concentrations, the calcium and magnesium content of *Symphysodon aequifasciata* vertebrae were 13.4 and 0.14 %, respectively, while in mineral-poor clear water, the calcium content of *S. discus* vertebrae was low (6.8 %) and partly compensated for by a high magnesium content (0.43 %). Referring to the extensive body armour of callichthyid catfish and the low mineral content of neotropical freshwaters, Luquet et al. (1991) called for a study of the calcium and phosphorus metabolism of armoured catfish.

Armoured catfish of the families Callichthyidae and Loricariidae are well represented in the fish faunas of neotropical rainforest creeks. The structure of the dermal plates has been described in detail (Bhatti, 1938; Sire and Meunier, 1993), but few data are available on the mineral content of the armour (Luquet et al., 1991) and no data are available on the mineral metabolism of the fish. The heavy bony armour may have an important function as a calcium and phosphate buffer (Herrmann-Erlee and Flik, 1989) and in protecting the fish against potential predators (Alexander, 1965; McLean and Godin, 1989), but its maintenance probably involves high energy costs because mineral accumulation must take place against steep electrochemical gradients. At least one callichthyid catfish (*Hoplosternum littorale*) does not survive in water with low ion concentrations and is restricted in its distribution to water with a relatively high mineral content (Mol, 1994). A large number of armoured catfish species are facultative air-breathers (Val and de Almeida-Val, 1995). Air-breathing may (Hulbert et al., 1978a) or may not (Hulbert et al., 1978b) have a major effect on the ion-regulatory function of the gill.

In the present study, we investigate the effect of ambient mineral concentrations on growth and on the accumulation of calcium, magnesium and phosphorus in newborn larvae of the callichthyid catfish *Megalechis personata* (Ranzani), which occur in mineral-poor black-water and clear-water creeks, but also in mineral-rich coastal swamps (Mol, 1994). Reis (1997) recognized a second species in *Hoplosternum thoracatum* (Cuvier and Valenciennes) and described a new genus to accommodate the two species: *Megalechis thoracata* (Cuvier and Valenciennes) and *M. personata* (Ranzani). According to Reis (1997), both *Megalechis* species occur in the coastal streams of the Guyanas (including Suriname). However, J. H. Mol and P. E. Ouboter (in preparation) provide evidence that only one species occurs in Surinam, *Megalechis personata*.

Megalechis is a continuous but facultative air-breather (Gee and Graham, 1978) that uses the intestine as an accessory respiratory organ (Huebner and Chee, 1978). However, the gills of *Megalechis* are well developed, and in hypoxic water *M. personata* can still respire aquatically at over 60 % of its routine rate of oxygen consumption in normoxic water (Gee and Graham, 1978). High rates of mineral accumulation may be expected in young growing fish, and this holds particularly for juvenile armoured catfish that are building up their bony

armour (Ballantyne, 1930). Net uptake of calcium, magnesium and phosphorus in the early life stages of *M. personata* was determined during an 8-week growth period in low- (rainwater) and high- (tapwater) mineral fresh water. The concentrations of calcium, magnesium and phosphorus in experimental fish were compared with the mineral content of young *M. personata* from natural habitats.

Materials and methods

Animals

Newborn free embryos (yolk-sac larvae) of *Megalechis personata* (Ranzani) (2.5 mg fresh mass; 6.5 mm total length) were used in the experiment. They were obtained by collecting naturally spawned eggs at the point of hatching from a swamp at Lelydorp, 20 km south of the capital Paramaribo (Suriname, South America), and then inducing hatching by exposing the eggs to water at 3–5 °C above the temperature of the swamp water. The larvae were transported to the laboratory in Paramaribo in oxygen-inflated polyethylene bags. After a 1-day adjustment period in tanks with mixed swamp water/tapwater (1:1), the larvae were distributed among the test tanks containing low- (LMF) and high- (HMF) mineral fresh water (Paramaribo rainwater and Paramaribo tapwater, respectively; for composition, see Table 1).

To compare the mineral concentrations of laboratory-reared *M. personata* with those of *M. personata* in their natural habitat, we collected juvenile *M. personata* using a small seine net (2.5 m×1.0 m; 2.0 mm unstretched mesh) in the Lelydorp swamp ($N=5$) and in a small rainforest creek 85 km south of Paramaribo (Maikaboeka Creek, Gros Rosebel area; $N=6$). Wild-caught *M. personata* were frozen at –15 °C, transported to Paramaribo on ice, and their length and fresh mass were determined immediately after arrival in the laboratory as described below.

Experimental protocol

The accumulation of calcium, magnesium and phosphorus was determined in early stages of *M. personata* maintained in rectangular glass tanks with a capacity of 75 l during an 8-week growth period. The experiment was first carried out in 1996 and repeated in 1997. Water temperature was not controlled during the experiment and ranged from 24 to 28 °C. The natural light period lasted from 07:00 to 19:00 h. Mortality was low (<10 %) and restricted to the first 2 weeks of the experiment, i.e. when the fish were very young. Fish samples for the determination of whole-body mineral concentrations and growth were taken from LMF and HMF tanks at weekly intervals and at 5 days after hatching.

Two days after hatching, the yolk sac had been consumed and the larvae started feeding on exogenous food. Larvae and juveniles were fed laboratory-reared *Artemia* nauplii twice daily at 07:00 and 17:00 h. The concentrations of calcium, magnesium and phosphorus in the *Artemia* nauplii were 16.0 ± 3.75 , 66.1 ± 1.30 and 359.9 ± 12.87 mmol kg⁻¹ (means \pm s.d., $N=10$; dry mass basis), respectively. The fish were kept

Table 1. Physico-chemical data for surface water in Suriname and test conditions in tanks during mineral accumulation experiments with *Megalechis personata* larvae compared with Amazonian rainforest streams near Manaus (Brazil)

	Lelydorp swamp, Suriname	Rainforest creeks, Suriname	HMF, Paramaribo tap water	LMF, Paramaribo rainwater	Amazonian streams*, near Manaus
Specific conductance at 25 °C ($\mu\text{S cm}^{-1}$)	99 \pm 47 (5)	29 \pm 2 (8)	1201 \pm 21 (16)	45.2 \pm 15.7 (15)	11 \pm 3 (20)
[Chloride] (mmol l^{-1})	0.34 \pm 0.20 (5)	0.13 \pm 0.01 (8)	7.86 \pm 0.42 (10)	0.152 \pm 0.093 (9)	0.062 \pm 0.011 (20)
pH	5.9 \pm 0.6 (5)	6.2 \pm 0.6 (8)	6.5 \pm 0.1 (16)	6.4 \pm 0.5 (15)	4.5 \pm 0.2 (20)
[Calcium] (mmol l^{-1})	0.12 \pm 0.06 (5)	0.019 \pm 0.005 (8)	0.59 \pm 0.005 (16)	0.073 \pm 0.026 (15)	0.0009 \pm 0.0008 (20)
[Magnesium] (mmol l^{-1})	0.085 \pm 0.042 (5)	0.020 \pm 0.006 (8)	1.94 \pm 0.057 (16)	0.015 \pm 0.006 (15)	0.0015 \pm 0.0006 (20)
[Phosphate] (mmol l^{-1})	<0.001 (5)	<0.001 (8)	<0.001 (2)	<0.001 (2)	<0.001‡ (20)
[Oxygen] (mg l^{-1})	— —	— —	4.0 \pm 1.3 (15)	5.2 \pm 0.7 (14)	— —

Values are means \pm S.D. (N).

*Data from Furch (1984); ‡total phosphorus.

HMF, high-mineral fresh water; LMF, low-mineral fresh water.

at densities of 6 fish l^{-1} (corresponding with a fresh mass of 30–200 mg l^{-1}) during the first 3 weeks of the experiment (1–21 days) and thereafter at densities of 2 fish l^{-1} (fresh mass 90–900 mg l^{-1} ; 22–56 days). To prevent the accumulation of metabolites and the growth of micro-organisms, half the water in each tank was changed twice daily before feeding. The water quality in the tanks was monitored with respect to dissolved oxygen levels, pH, conductance and concentrations of chloride, calcium, magnesium and phosphate.

Analytical procedures

Water analyses of natural water bodies and of rainwater and tapwater in the tanks followed standard methods (Greenberg et al., 1995). Dissolved oxygen, specific conductance and pH were determined immediately after sampling using a YSI dissolved oxygen meter (model 50B), a YSI conductometer (model 35) and a WTW microprocessor meter (model 539), respectively. Water samples for the determination of chloride and phosphate levels were stored at 4 °C until the time of analysis. Water samples for the analysis of calcium and magnesium levels were acidified to pH < 3 with known volumes of concentrated HNO_3 and stored in polyethylene bottles at room temperature. Calcium and magnesium concentrations were determined by Suralco Aluminum Company (Suriname) with a Fisons (ARL) simultaneous ICP apparatus.

The fresh mass of the samples was determined to the nearest 0.1 mg after blotting the fish on damp filter paper. The fish were killed by snap-freezing and then dried for 24 h at 70 °C. Their dry mass was determined to the nearest 0.1 mg, and the dried samples were dissolved in a dry-mass-determined volume of concentrated HNO_3 at room temperature for 4 days. Adding dried fish material to the HNO_3 increased the volume of the solution by 1 ml per 5 g fish sample, and this increase in volume

was taken into account in all further calculations. The fish digests were diluted 100 \times with distilled water, and total calcium, magnesium and phosphorus were determined using an ICP apparatus at Nijmegen University (The Netherlands). The calcium, magnesium and phosphorus contents of fertilized eggs of *M. personata* and of the *Artemia* nauplii used as food were determined according the procedures described above for fish samples.

Calculations and statistics

The accumulation of calcium, phosphorus and magnesium in LMF and HMF test conditions was compared by (1) calculating the mean mineral influx ($\Delta Q_f/\Delta t$) and (2) establishing the linear regression equation $Q_f = a + bW_f$, where Q_f ($\mu\text{mol fish}^{-1}$) is mineral content and W_f (mg fish^{-1}) is dry mass. Differences in slopes of the Q_f regression lines were analyzed using a test of equality of slopes (Sokal and Rohlf, 1995). Student's *t*-test was used to assess the difference in the dry mass of the fish in LMF and HMF at the end of the experiment. Data are presented as mean values \pm 1 standard deviation (S.D.). Significance was accepted when $P < 0.05$.

Results

Concentrations of calcium, magnesium and phosphorus in water

The mineral concentrations of the natural fresh waters of Suriname differ greatly. In the rainforest creeks that drain the Precambrium Guyana shield, *M. personata* occurs in water with a low mineral content comparable with that of Paramaribo rainwater (Table 1). *Megalechis personata* also occurs in rainforest streams near Manaus in Brazil (Reis, 1997), where mineral concentrations are extremely low (Table 1; Furch,

1984). In the swamps of the coastal plain (e.g. the Lelydorp swamp), *M. personata* occurs in water with higher mineral concentrations (Table 1). In the dry season, mineral concentrations of coastal swamps are comparable with those of Paramaribo tapwater (Mol, 1994). The two types of water used in the experiments, LMF (rainwater) and HMF (tapwater), have calcium, magnesium and phosphate concentrations that are well within the range of the concentrations of these minerals in the natural habitats of *M. personata* (Table 1; Mol, 1994).

Growth and development

Growth of the *M. personata* larvae was rapid both in 1996 and in 1997 (Fig. 1). The increase in mass was exponential during the 56-day experiment. The dry masses of *M. personata* at the end of the experiment differed significantly between LMF and HMF in both the 1996 and 1997 experiments (Fig. 1). However, the difference in growth was not consistent: when comparing the dry mass at the end of the experiment in LMF and HMF water, *M. personata* grew faster in HMF than in LMF in 1996 ($P < 0.001$), whereas the fastest growth was observed in LMF ($P < 0.001$) in 1997. At the end of the experiment, juveniles measured 3.4–4.0 cm total length and weighed 0.5–1.4 g fresh mass (0.11–0.34 g dry mass).

The larvae used branchial respiration initially. At the age of approximately 2 weeks, the larval finfold had disappeared, all the fins were fully developed and the fish began to surface for air. Thus, at the start of the juvenile stage, the fish began to employ bimodal respiration. Approximately 1 month after hatching, the two longitudinal rows of bony plates were beginning to form on each side of the body. The scutes grew from the horizontal septum in either the dorsal or ventral direction. However, at the end of the experiment, the lateral

bony armour still did not cover the whole body. Fully grown adult *M. personata* weigh approximately 100 g (35 g dry mass) and measure 17.0 cm in total length; the bony parts of the head armour and lateral scutes make up 20–30 % of the body fresh mass (the dry mass of the armour is 55–65 % of the body dry mass).

Accumulation of calcium, phosphorus and magnesium in LMF and HMF test conditions

The calcium, phosphorus and magnesium contents of fertilized eggs of *M. personata* were 0.434 ± 0.029 , 0.839 ± 0.054 and 0.147 ± 0.013 % ($N=10$; dry mass basis), respectively. When comparing the 1996 and 1997 experiments, patterns of mineral accumulation were consistent in LMF (rainwater), but in HMF (tapwater) the accumulation of calcium and phosphorus showed minor differences during the first 3 weeks after hatching.

In LMF, the calcium content of young *M. personata* increased rapidly from 0.7 % (dry mass basis) at hatching to approximately 2.5 % at 28 days and then increased more gradually to 3.2 % in the second month of growth (Fig. 2A). In the HMF experiment, the accumulation of calcium showed a slight reduction in the calcium content from 0.7 % to 0.5 % ($P < 0.001$) in the first week after hatching, followed by an increase in calcium content to approximately 1.3 % at 28 days and then to 1.7 % at the end of the experiment (Fig. 2A). The slight reduction in calcium accumulation did not occur in the HMF experiment of 1996, but the calcium content at the end of this experiment was also 1.7 %.

The phosphorus content in LMF started at a higher level (1.7 %) than that of calcium and reached a stable value of approximately 2.4 % at an early age (21 days, Fig. 2B). In HMF, the phosphorus content showed a slight reduction from 1.7 % at hatching to 1.5 % in the first week of the experiment ($P=0.41$; the reduction was absent in the 1996 experiment), but there was no further significant increase in the phosphorus content of the fish, and the final value was approximately 1.6 % (Fig. 2B). The calcium/phosphorus ratio increased from 0.35 at hatching to approximately 1.1 in LMF and 0.8 in HMF (Fig. 2D).

In both LMF and HMF, the magnesium content of *M. personata* was 0.15 % throughout the experimental period (Fig. 2C), i.e. equal to the magnesium content of fertilized eggs.

When calculating the mean rate of mineral influx and Q_f regression lines for mineral accumulation in *M. personata*, the period of accelerated calcium and phosphorus accumulation (the first 3 weeks after hatching) was excluded from the analysis. The mean net rate of calcium influx was 11.8 – $15.7 \mu\text{mol day}^{-1} \text{g}^{-1}$ fish in LMF and 6.1 – $10.2 \mu\text{mol day}^{-1} \text{g}^{-1}$ fish (fresh mass basis) in HMF (Table 2). Calcium was accumulated approximately twice as fast in LMF as in HMF (Table 2; Fig. 3A); in both the 1996 and 1997 experiments, the slopes of the Q_f -regression lines were significantly different between LMF and HMF ($P < 0.01$). The calcium content of juvenile *M. personata* from natural habitats followed the LMF line (Fig. 3A). The net rate

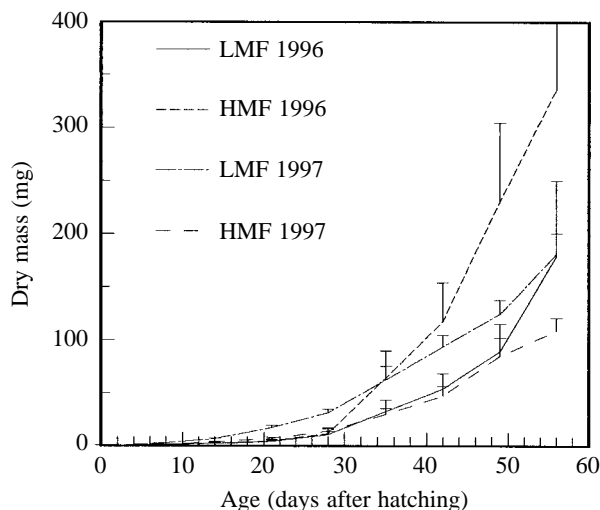


Fig. 1. Mean dry mass (+ s.d.) for independent groups of 10 larvae/juveniles of *Megalechis personata* in low-mineral fresh water (LMF) and high-mineral fresh water (HMF) test conditions during two experiments (1996 and 1997). In 1996, *M. personata* grew significantly faster in HMF than in LMF, whereas in 1997 the fastest growth was observed in LMF.

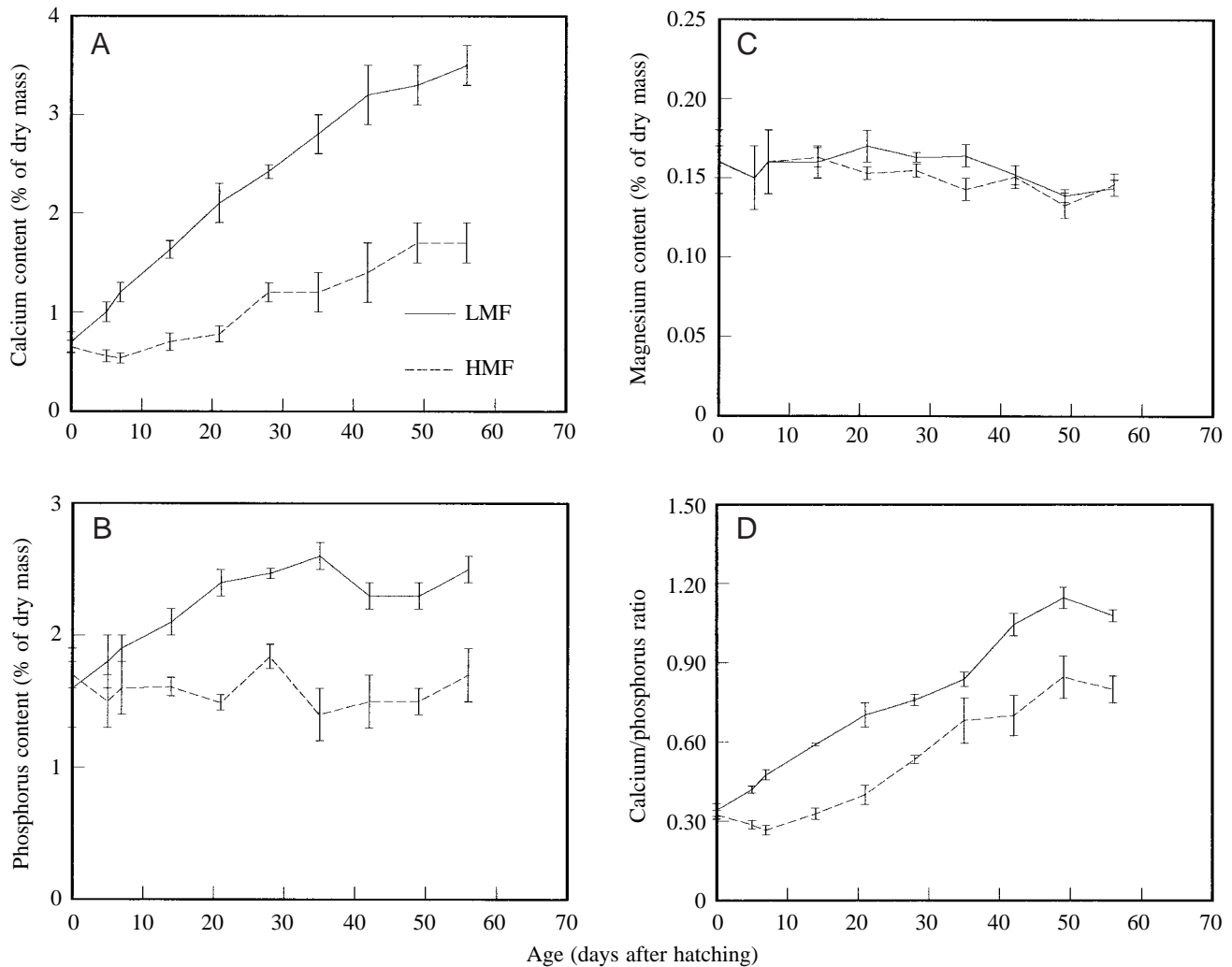


Fig. 2. Mean whole-body content of calcium (A), phosphorus (B) and magnesium (C) and calcium/phosphorus ratio (D) for independent groups of 10 larvae/juveniles of *Megalechis personata* grown in low-mineral fresh water (LMF) and high-mineral fresh water (HMF) test conditions during the 1997 experiment (means \pm S.D.).

of phosphorus influx was $11.2\text{--}14.5\ \mu\text{mol day}^{-1}\ \text{g}^{-1}$ fish in LMF and $7.7\text{--}11.7\ \mu\text{mol day}^{-1}\ \text{g}^{-1}$ fish in HMF (Table 2). The accumulation of phosphorus in LMF was approximately 1.5 times faster than the accumulation of phosphorus in HMF (Table 2; Fig. 3B); in both the 1996 and 1997 experiments, the slopes of the regression lines differed significantly ($P < 0.01$). The phosphorus content of wild-caught juveniles followed the LMF accumulation line (Fig. 3B). The net rate of magnesium influx was $1.3\text{--}1.4\ \mu\text{mol day}^{-1}\ \text{g}^{-1}$ fish in LMF and $1.0\text{--}1.3\ \mu\text{mol day}^{-1}\ \text{g}^{-1}$ fish in HMF (Table 2). The rate of accumulation of magnesium did not differ between LMF and HMF (Table 2; Fig. 3C), and the slopes of the regression lines did not differ from each other in both 1996 and 1997. The magnesium content of juveniles from natural habitats followed the LMF and HMF lines (Fig. 3C).

Discussion

During the first 8 weeks after hatching, young *M. personata*

showed a net uptake of calcium, magnesium and phosphorus as indicated by the accumulation of these elements in the body. A net uptake of calcium, magnesium and phosphorus was observed in HMF and also in LMF, in which there was an eightfold reduction of ambient calcium level ($0.073\ \text{mmol l}^{-1}$) and a 130-fold reduction of ambient magnesium level ($0.015\ \text{mmol l}^{-1}$). In fact, calcium accumulated twice as fast and phosphorus 1.5 times as fast in LMF as in HMF, while the rate of accumulation of magnesium did not differ between LMF and HMF. The differences in the rates of accumulation of calcium and phosphorus between LMF and HMF were independent of differences in growth rate between LMF and HMF in both the 1996 and 1997 experiments. An increased rate of calcium uptake in low-calcium fresh water was also observed in the killifish *Fundulus heteroclitus* (Mayer-Gostan et al., 1983) and in young tilapia *Oreochromis mossambicus* (Flik et al., 1986), but in goldfish *Carassius auratus* (Berg, 1968, 1970), rainbow trout *Oncorhynchus mykiss* and bullhead catfish *Ictalurus nebulosus* (Höbe et al., 1984), the net rate of

Table 2. Comparison of the accumulation of calcium, magnesium and phosphorus in the early life stages of *Megalechis personata* in LMF and HMF test conditions and in the 1996 and 1997 experiments

Mineral	Experiment	Net mineral influx ($\mu\text{mol day}^{-1} \text{g}^{-1}$)	Parameters of Q_f regression line			
			a	b	r^2	N
Calcium	1996 LMF	15.7 \pm 3.9 (5)	-1.74	0.840	0.9268	59
	1996 HMF	10.2 \pm 7.0 (5)	0.97	0.445	0.8480	60
	1997 LMF	11.8 \pm 2.1 (5)	-9.58	0.911	0.9882	60
	1997 HMF	6.1 \pm 1.9 (5)	-3.20	0.458	0.9616	59
Magnesium	1996 LMF	1.4 \pm 0.6 (9)	0.33	0.063	0.9295	98
	1996 HMF	1.3 \pm 0.8 (9)	0.55	0.054	0.9415	100
	1997 LMF	1.3 \pm 0.7 (9)	0.11	0.060	0.9954	100
	1997 HMF	1.0 \pm 0.3 (9)	0.06	0.058	0.9908	99
Phosphorus	1996 LMF	14.5 \pm 4.8 (5)	3.21	0.691	0.9219	59
	1996 HMF	11.7 \pm 8.5 (5)	7.21	0.450	0.9137	60
	1997 LMF	11.2 \pm 4.4 (5)	-0.87	0.791	0.9877	60
	1997 HMF	7.7 \pm 2.3 (5)	-0.60	0.525	0.9752	59

Net mineral influx ($\mu\text{mol day}^{-1} \text{g}^{-1}$ fish, fresh mass basis) and parameters of the linear regression analysis for $Q_f = a + bW_f$ are given. Data of the first 3 weeks after hatching (0, 5, 7 and 14 days) are excluded from the analysis of the accumulation of calcium and phosphorus. Number of observations in parentheses.

Q_f , mineral content ($\mu\text{mol fish}^{-1}$); W_f , dry mass (mg fish^{-1}).

Values are means \pm S.D. (N).

HMF, high-mineral fresh water; LMF, low-mineral fresh water.

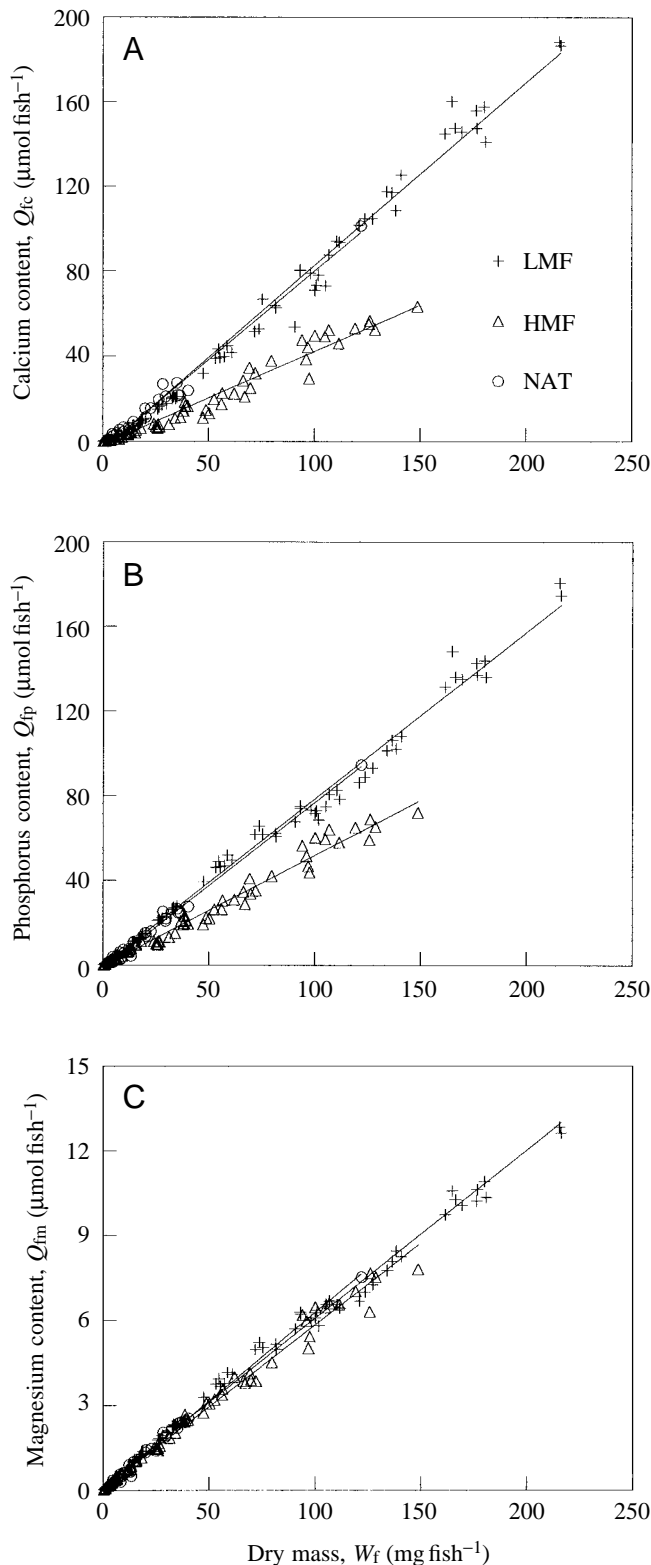
calcium uptake was largely independent of ambient Ca^{2+} levels.

The increased rate of calcium accumulation in *M. personata* in LMF was independent of the different growth performance (food intake) in the 1996 and 1997 experiments (Fig. 1), suggesting that calcium was taken up mainly through the integumental ionocytes. In tilapia (*O. mossambicus*) acclimated to low ambient calcium levels, branchial uptake could account for 70 % of the accumulated calcium (Flik et al., 1986).

Although linear extrapolation of the rates of mineral accumulation by early life stages of *M. personata* to rates of accumulation for a (hypothetical) fish of 1 kg may be misleading (Flik et al., 1986), it allows at least a crude comparison of our data with those published in the literature. The net calcium accumulation rate of $254 \mu\text{mol h}^{-1} \text{kg}^{-1}$ (HMF, 1997) to $654 \mu\text{mol h}^{-1} \text{kg}^{-1}$ (LMF, 1996) in early stages of *M. personata* are 12.5 times and 6.5 times, respectively, higher than net rates of accumulation for young tilapia in fresh water (approximately $20 \mu\text{mol h}^{-1} \text{kg}^{-1}$) and low-calcium fresh water (approximately $100 \mu\text{mol h}^{-1} \text{kg}^{-1}$; Flik et al., 1986). Rates of accumulation of calcium in goldfish (Berg, 1968, 1970), rainbow trout and bullhead catfish (Höbe et al., 1984) and killifish (e.g. Mayer-Gostan et al., 1983) are in the range $1\text{--}50 \mu\text{mol h}^{-1} \text{kg}^{-1}$. The high rates of calcium accumulation in young *M. personata* may to some extent represent an overestimation resulting from the extrapolation to 1 kg fish

(Flik et al., 1986), but they probably also reflect high actual rates of mineral accumulation during exponential growth in early developmental stages while the fish are building their dermal armour. The high rate of calcium accumulation and the lack of a discontinuity in the calcium accumulation curves after the transition from aquatic respiration to bimodal respiration in the third week after hatching suggest that air-breathing did not have a major effect on ion regulation in young *M. personata*. Adult *Megalechis* have an efficient aquatic respiratory system, and their accessory respiratory organ (intestine) has also assumed a hydrostatic function (Gee and Graham, 1978). The slight reduction in the whole-body calcium content of *M. personata* in HMF test conditions during the first 2 weeks of the 1997 experiment may be related to stress resulting from the transfer of newborn larvae from low-mineral swamp water to high-mineral tapwater. Newborn carp larvae have a fully functional stress response, including elevated cortisol levels (Stouthart et al., 1998). The hormone cortisol plays an important role in ion regulation in freshwater fish (Flik and Perry, 1989; Wendelaar Bonga, 1997).

Rates of accumulation of magnesium in young *M. personata* did not differ between LMF (magnesium $0.015 \text{ mmol l}^{-1}$) and HMF conditions, and the magnesium content of the fish (0.15 %) was regulated within narrow limits throughout the 8-week experiment. Carp embryos require a critical magnesium level in the ambient water of 0.01 mmol l^{-1} for survival (van der Velden et al., 1991a). The steady magnesium content of



early stages of *M. personata* in both LMF and HMF and at different ages (Fig. 2C) suggests that the low magnesium concentration of LMF water did not pose a problem for magnesium uptake by embryos and yolk-sac larvae. Thus, with respect to the uptake of calcium and magnesium, *M. personata* is apparently well adapted to neotropical fresh waters with an

Fig. 3. Accumulation of calcium (A), phosphorus (B) and magnesium (C) in the early life stages of *Megalechis personata* in low-mineral fresh water (LMF) and high-mineral fresh water (HMF) test conditions during the 1997 experiment and in natural habitats in Suriname (NAT). Data for the first 3 weeks of the experiment were excluded from the calculation of the regression lines $Q_f = a + bW_f$, where Q_f ($\mu\text{mol fish}^{-1}$) is the mineral content and W_f (mg fish^{-1}) is dry mass, for the minerals calcium and phosphorus. Details of the regression lines are given in Table 2. The slopes of the Q_f regression lines differed significantly between LMF and HMF for calcium and phosphorus ($P < 0.01$).

extremely low mineral content. Young freshwater fish do not rely exclusively on absorption of magnesium from the water via the gills, but also take up magnesium from the food through the intestinal wall (van der Velden et al., 1990). The magnesium concentration of the *Artemia* nauplii ($66.1 \text{ mmol kg}^{-1}$) used in our experiments was high compared with the magnesium requirement of 20 mmol kg^{-1} food for optimal growth in small carp (Ogino and Chiou, 1976). The rate of magnesium accumulation in the early stages of *M. personata* ($41.7\text{--}58.3 \mu\text{mol h}^{-1} \text{ kg}^{-1}$) is high compared with the growth-related rate of magnesium uptake of $2.15 \mu\text{mol h}^{-1} \text{ kg}^{-1}$ of young tilapia *Oreochromis mossambicus* (van der Velden et al., 1991b).

Phosphate was not present in measurable quantities in either HMF (tapwater) or LMF (rainwater) (Table 1). Although, in fish, phosphorus is usually taken up by the intestinal epithelium from the food (Herrmann-Erlee and Flik, 1989), the rate of accumulation of phosphorus in the early stages of *M. personata* differed significantly between LMF and HMF. Although food was supplied *ad libitum* to the fish in all experiments, the difference in growth performance between LMF and HMF conditions was not consistent when the experiments of 1996 and 1997 are compared. It follows that the 1.5-fold increase in the rate of phosphorus accumulation in LMF (compared with HMF) could not be accounted for by an increase in the food uptake as such, but only by an increased selective uptake of phosphorus from the food. The 1.5-fold and twofold increases in the rates of accumulation of phosphorus and calcium, respectively, in LMF suggest a close relationship between the uptake of phosphorus from the food and of calcium from the water in *M. personata*. It is tempting to speculate that the accumulation of calcium is critical in determining the uptake of phosphorus or, *vice versa*, that the uptake of calcium follows the accumulation of phosphorus, because strict regulation of both is indicated by steadily increasing calcium/phosphorus ratios.

While the mineral composition of the vertebrae of two species of discus fish *Symphysodon* reflected a difference in the mineral content of the water in which they live (Geisler and Schneider, 1976), the calcium and magnesium concentrations of juvenile *M. personata* did not reflect the concentrations of these minerals in the water in which they were raised (e.g. the higher calcium content in LMF fish). The mineral content of juvenile *M. personata* may be compared with the mineral content of adults of the related catfish *Hoplosternum littorale* (Luquet et al., 1991). Not surprisingly, the calcium (3.2%) and

phosphorus (2.4 %) contents and the calcium/phosphorus ratio (1.1) of 8-week-old *M. personata* were low compared with the calcium (8.4 %) and phosphorus (4.2 %) contents and calcium/phosphorus ratio (2.0) of adult *H. littorale*. However, it is remarkable that the magnesium content of the eggs and early stages of *M. personata* (0.15 %) did not differ greatly from the magnesium content of adult *H. littorale* (0.2 %; Luquet et al., 1991). While calcium and phosphorus have specific storage depots (bone tissue), magnesium is not so easily stored.

The calcium and phosphorus contents of wild-caught juvenile *M. personata* reflected the pattern of fast mineral accumulation in LMF test conditions. The mineral concentrations in the Lelydorp swamp and the Maikaboeka rainforest creek are more similar to the mineral levels in LMF (rainwater) than to the mineral concentrations in HMF (tapwater) (Table 1). Calcium and phosphorus are the major mineral constituents of bone (Herrmann-Erlee and Flik, 1989). In LMF-raised tilapia, the bone compartment provides an important, readily exchangeable, calcium pool and may act as a calcium buffer under conditions of low ambient calcium levels (Flik et al., 1986). In *M. personata*, the accumulation of calcium and phosphorus is also critical for the formation of the bony armour that protects the fish against predators (Lowe-McConnell, 1987; McLean and Godin, 1989; but see Andraso and Barron, 1995). Neotropical fresh waters are well known for their large numbers of predators of fish (Lowe-McConnell, 1987; Winemiller, 1989; Mol, 1996). Predation pressure on *M. personata* is greatest during the early developmental stages, but the vulnerability of individual *M. personata* to small-sized predators decreases rapidly with increased body size (Mol, 1996). The body armour may be more effective in protecting adult *M. personata* against large piscivores, e.g. by reducing the palatability of the fish (as suggested by Lowe-McConnell, 1987). In Surinam, adult armoured catfish (both callichthyids and loricariids) are often recovered undamaged from gill nets, while naked catfish and characins are heavily preyed upon by piranhas (J. H. Mol, personal observation). The piscivore *Hoplias malabaricus* is one of the most important predators of adult *M. personata* (Mol, 1996). In a laboratory experiment, *H. malabaricus* (25 cm standard length, SL) preferred the cichlid *Crenicichla saxatilis* (11.4–12.0 cm SL; no armour, no pectoral spines) to the naked catfish *Parauchenipterus galeatus* (11.2–12.4 cm SL; no armour, pectoral spines present), while *M. personata* (11.2–13.6 cm SL; armour and pectoral spines present) was taken only when no other prey was available (J. H. Mol and H. Bouwmeester, unpublished data).

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