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Research Article

Growth, elemental and proximate biochemical composition of larval Amazon River prawn, *Macrobrachium amazonicum*, reared under different salinity conditions

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ABSTRACT. In the aquaculture of prawns in inland facilities, the supply with natural seawater is technically difficult and expensive, while the use of artificial salt may be suboptimal due to unfavorable ionic composition. In the present study, Amazon River prawn, *Macrobrachium amazonicum*, were reared from hatching through nine larval stages to the first juvenile instar, comparing four experimental conditions with two salinities (5, 10) and two different types of salt (artificial, natural). Larval biomass growth was measured in terms of changes in dry weight (W), contents of carbon and nitrogen (C, N), and proximate biochemical composition (lipid, protein); moreover, body size (carapace length, CL) was measured in first-stage juveniles. After passing through the non-feeding first larval stage, later stages showed an exponential increase in values of biomass per individual. Rates of increase differed significantly among treatments, showing generally lower growth in experiments with artificial *vs.* natural salt, and at 5 *vs.* 10. The same response pattern was found also in CL of early juvenile shrimps. Similar but mostly weaker effects were observed in the percentage C, N, lipid, and protein values (in % of W), and in the C: N mass ratio. Our data indicate that larval rearing of *M. amazonicum* is feasible with artificial salts and at lower than commonly used standard salinity (10). This makes the cultivation of this species feasible also in aquaculture facilities located at large distance from the coast, where a reduction of costs and logistic investments may compensate for reduced larval growth and production of smaller juveniles. However, these salinity effects on offspring production have to be taken into account in comparisons of growth data from different laboratories and locations.

Keywords: *Macrobrachium amazonicum*, rearing, larval growth, development, lipid, protein, aquaculture.

INTRODUCTION

The Amazon River prawn, *Macrobrachium amazonicum* (Heller, 1862), is a widely distributed species of palaemonid shrimp in the Amazon and Orinoco basins, living mostly in shallow lagoons and rivers near estuaries in northern and northeastern Brazil, the Guianas, and Venezuela (Holthuis, 1952; López-Sánchez & Pereira, 1998; for recent revision of taxonomic status and biogeography, see dos Santos *et al.*, 2013). Due to its large body size and rapid growth, it is a target species of regional coastal fisheries and a promising candidate for commercial aquaculture (New, 2005; Maciel & Valenti, 2009; Hayd *et al.*, 2010, 2014; Moraes-Valenti & Valenti, 2010).

Although adult populations of *M. amazonicum* live mostly in limnic habitats, the larval stages show little freshwater tolerance and only limited capabilities of hyper-osmoregulation (Charmantier & Anger, 2011; Boudour-Boucheker *et al.*, 2013, 2016). This indicates a diadromous strategy with larval export to salt water, followed by larval development in estuarine or coastal marine water, and finally an upstream migration and recolonization of limnic habitats by juveniles (for recent review of reproductive strategies in freshwater shrimp, see Bauer, 2013). Since unfavorable osmotic

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conditions may cause enhanced metabolic losses, and thus, reduced larval survival and growth (McNamara *et al.*, 1983; Zanders & Rodríguez, 1992; Huong *et al.*, 2010), this raises the question which salinities allow for successful rearing in research cultivation and commercial aquaculture.

Development from hatching to early juvenile stages of *M. amazonicum* is possible at oligohaline conditions (salinities 1-5), but maximum survival has been observed at higher salinities (10-16; Guest & Durocher, 1979; Barreto & Soares, 1982; Araujo & Valenti, 2007). Besides total salt concentration, also the ionic composition of the culture water is important for physiological functions such as respiration, excretion, and osmoregulation, especially at low salinities (Roy *et al.*, 2007). This is relevant for aquaculture in inland facilities, where natural seawater supply is logistically very difficult and expensive, while the use of artificial salt may be suboptimal due to unfavorable ionic composition. However, no comparative studies of larval growth in natural and artificial salt water have been available for *M. amazonicum*.

In the present experimental laboratory study, larvae of *M. amazonicum* originating from the upper Amazon estuary were reared at two salinities (5, 10), comparing natural and artificial sea salt. Larval growth was quantified in terms of changes in dry mass (W), elemental (C, N) and proximate biochemical composition (protein, lipid), as well as body size (carapace length, CL) of first-stage juveniles.

MATERIALS AND METHODS

Origin and maintenance of prawns

Adult *M. amazonicum* were obtained from the Aquaculture Center of the State University of São Paulo, Jaboticabal, Brazil (CAUNESP; for details of broodstock production and maintenance, see Moraes-Valenti & Valenti, 2007). The broodstock originated from estuarine tidal creeks near the city of Belém in the Amazon Delta in northern Brazil (01°14'30"S, 48°19'52"W), close to the type locality of this species (W. Valenti, *pers. comm.*). Male and ovigerous female shrimps were transported by air freight from Brazil to the Helgoland Marine Biological Laboratory (BAH), Germany, maintained in cooling boxes with aerated fresh water. The ovigerous females were subsequently transferred to recirculating aquaria provided with 30 L aerated freshwater (total ion concentration <0.2 mg L^{-1}) and gravel filters, kept at a temperature of 29 ± 1°C, a 12:12 h light:dark cycle, and fed with pieces of frozen marine isopods (*Idotea* spp.) and commercial aquarium feeds (Novo Tab, JBL; Anger *et al.*, 2009). Sieves (0.3 mm mesh size) receiving the overflowing water from the aquaria were checked daily for the appearance of newly hatched larvae.

Larval rearing

Actively swimming newly hatched larvae from four females were transferred to aerated beakers with 1 L filtered water and subsequently reared under four experimental conditions with two salinities (5, 10) and two different types of salt (artificial *vs.* natural). Different salinities were obtained by mixing appropriate amounts of tap water (total ion concentration 0.2 mg L^{-1}) with natural seawater from the southern North Sea (salinity *ca.* 33) or with refined industrial sea salt (Tropic Marin salts; Wartenberg, Germany), respectively. Salinities were measured to the nearest 0.1 using a temperature-compensated electric probe (WTW Cond 330i, Weilheim, Germany). Conditions of temperature and light were the same as in the maintenance of adult shrimps. The cultures were checked every 24 h for molts or mortality when water and food (freshly hatched brine shrimp nauplii, *Artemia franciscana*; Great Salt Lake stock; *ca.* 10-15 nauplii mL^{-1}) were changed. A recent study revealed that a daily feeding interval and high food density (4-12 nauplii mL^{-1}) allow for optimal larval survival and growth (Maciel *et al.*, 2014). No food was given to the first zoeal stage because it is fully lecithotrophic (Anger & Hayd, 2009).

Sampling, measurements of dry weight (W), elemental (C, N) and proximate biochemical composition (total lipid, protein)

The larval development of *M. amazonicum* shows individual variability in the number and duration of successive larval instars, normally comprising 8-10, occasionally up to >12 molts, before the first juvenile stage is reached *ca.* 20-22 days after hatching (Anger *et al.*, 2009). The change from late larval to early juvenile stages is in this species, typically for caridean shrimps, a gradual transition without metamorphosis (Anger, 2001). The first juvenile was here defined as the first postembryonic stage where larval traits (*e.g.*, natatory exopods on the pereiopods; planktonic swimming behavior) become functionally insignificant, while juvenile-adult traits clearly predominate (*e.g.*, benthic crawling behavior, use of pereiopodal endopods as walking legs) (Anger, 2001). In the present study, we quantified larval growth under different experimental conditions only for the most commonly observed developmental pathway, which consisted of nine larval stages followed by a juvenile. All larvae were therefore microscopically staged and sorted in daily intervals so that each rearing beaker (n = 4) contained homogeneous material with individuals that molted the same day to the same morphological stage; in each treatment, developmentally retarded larvae were removed from

cultures and not used for analyses. The average time of molting to successive stages was slightly delayed in treatments with low salinity (5) and artificial salt in comparison to salinity 10 and natural sea salt, reaching in total about two days at the end of the experiment. This slight delay was not quantified in this study; likewise, no mortality rates were determined, as survival was generally high (>80%).

Each successive stage was sampled one day after molting; this implies that the time of sampling was determined by the time after each molt (stage C of the molting cycle; Hayd *et al.*, 2008) and not by the absolute age from hatching. For each sample, parallel determinations of dry weight (W) and contents of carbon (C), nitrogen (N), total lipid, and protein were carried out with standard techniques (Urzúa & Anger, 2011; Urzúa *et al.*, 2012), considering these constituents as proxies of organic matter. Moreover, carapace length (CL) was microscopically measured in first-stage juveniles, indicating the increment in body size achieved during the period of total larval development. All measurements of W, C and N comprised five replicate samples with 2-5 individuals each, depending on individual W. For proximate biochemical analyses, four replicate determinations were carried out with 12-15 individuals each.

Statistical analyses

Statistical analyses were performed with standard methods (Sokal & Rohlf, 1995; Quinn & Keough, 2002) using the software package Statistica 8 (StatSoft). Differences in W, C, N, lipid, and protein (response variables) in different developmental stages were tested using a 4-way ANOVA (nested design) with "Salinity" and "Type of salt" as independent factors, and "Female" and larval "Stage" as nested factors. Differences in body size (CL) of first-stage juveniles were tested using a two-way ANOVA with salinity and type of salt as factors. Where significant differences occurred, these were further analyzed with multiple pairwise comparison tests [*Post-hoc* test Student-Newman-Keuls (SNK tests)]. All tests were run on the 95% confidence level ($P < 0.05$). Normality and homogeneity of variances were tested with Kolmogorov-Smirnov and Levene tests, respectively.

RESULTS

Larval growth (biomass and body size per individual)

The first two zoeal stages of *M. amazonicum* are fully or facultative lecithotrophic, respectively (see Anger & Hayd, 2009). Compared with the Zoea-I stage (hatched in fresh water and sampled at the beginning of larval exposure to different experimental treatments), the Zoea II (1 day after molting) showed, therefore, no growth, regardless of salinity and type of salt (Table 1). Subsequent developmental instars, by contrast, showed in all measures of biomass per individual, and in all treatments, exponential patterns of increase (Table 1).

The rate of growth tended to be slightly lower in treatments with low salinity (5 *vs.* 10) and in those with artificial *vs.* natural salt. Statistically significant differences between treatments were more consistently found in dry weight (W) and amounts of elemental constituents per individual (C, N; Table 1) than in biochemical parameters (lipid, protein; Table 1). In the latter, effects of salinity were generally more conspicuous than those of the type of salt.

The tendencies observed in larval growth under different experimental conditions were clearly visible also in differential body size of first-stage juveniles (Table 2). Mean carapace length (CL) was significantly smaller after larval rearing at salinity 5 compared to 10; at both salinities, it was significantly smaller in treatments with artificial instead of natural salt. Among the conditions tested here, a combination of low salinity (5) with artificial salt (treatment 5A) was pessimum, while salinity 10 prepared by dilution of natural seawater (treatment 10N) represented the optimum among the rearing conditions tested here.

In spite of being statistically significant, the reduction of larval growth under suboptimal conditions was in general small. When natural sea salt was used, a reduction of salinity alone (treatment 5N *vs.* 10N) caused a reduction in early juvenile size by 11%. At salinity 10, the use of artificial salt reduced the CL by 8% (treatment 10A *vs.* 10N). The maximum reduction in body size (19%) was observed under pessimum compared to optimum rearing conditions (treatment 5A *vs.* 10N).

Changes in elemental and proximate biochemical composition

Compared to values of biomass per larva, those of relative elemental and biochemical composition (in % of W; C/N mass ratio) revealed less clear patterns of developmental change (Table 3). The percentage C content and the C/N ratio decreased initially (larval stages I-V), remained subsequently rather stable, and increased slightly in late larval stages and the first juvenile. The N content showed a weak tendency to increase during early development (larval stages I-V), remaining rather stable thereafter.

The percentage values (in % of W) of C and the C/N mass ratio tended to be lower in treatments with artificial *vs.* natural salt and at salinities 5 *vs.* 10. By contrast, the N content was mostly maximum in the treatment with artificial salt and salinity 5, without showing a consistent tendency in the other treatments.

Table 1. *Macrobrachium amazonicum*, larval biomass growth (all values in µg ind^{-1}; mean values ± SD): changes in dry weight (W), carbon (C), nitrogen (N), lipid and protein content of successive developmental stages reared in four experimental treatments (Exp.) with combinations of two salinities (5, 10) and two different types of salt (A: artificial, N: natural); different numbers of decimals reflect differential variability among replicate measurements (no decimals given for W and for values >100 µg); different lower case letters indicate significant differences between treatments (same developmental stage); asterisks indicate significant differences to previous stage (same treatment); SNK tests.

Biomass (µg ind^{-1})	Exp.	Stage									
		I	II	III	IV	V	VI	VII	VIII	IX	X
W	5A	63.1 ± 2.4	72 ± 2^{a*}	91 ± 6^{a*}	99 ± 4^{a*}	151 ± 6^{a*}	297 ± 2^{a*}	334 ± 32^{a*}	324 ± 41^{a*}	456 ± 47^{a}	474 ± 57^{a}
	5N		71 ± 2^{a*}	89 ± 9^{a*}	127 ± 6^{b*}	170 ± 9^{b*}	255 ± 13^{b*}	291 ± 45^{a*}	412 ± 31^{b*}	490 ± 41^{b*}	607 ± 62^{b*}
	10A		75 ± 1^{a*}	103 ± 5^{b*}	102 ± 14^{a*}	136 ± 26^{a*}	279 ± 21^{c*}	371 ± 45^{a*}	420 ± 49^{b*}	482 ± 49^{b*}	638 ± 94^{b*}
	10N		75 ± 2^{a*}	107 ± 3^{b*}	138 ± 9^{c*}	213 ± 8^{c*}	318 ± 14^{d*}	452 ± 40^{b*}	537 ± 31^{c*}	580 ± 32^{c*}	620 ± 50^{b*}
C	5A	34.3 ± 1.6	34.5 ± 1.3^{a*}	43.8 ± 2.1^{a*}	43.6 ± 1.8^{a*}	66.6 ± 3.0^{a*}	130 ± 9^{a*}	144 ± 16^{a*}	144 ± 21^{a*}	193 ± 21^{a*}	202 ± 27^{a}
	5N		34.9 ± 0.8^{a*}	43.1 ± 3.1^{a*}	56.2 ± 2.3^{b*}	75.1 ± 4.3^{b*}	109 ± 7^{b*}	128 ± 19^{a*}	185 ± 16^{b*}	227 ± 21^{b*}	273 ± 33^{b*}
	10A		36.4 ± 0.5^{a*}	47.2 ± 2.3^{b*}	45.2 ± 6.1^{a*}	57.0 ± 11.3^{a*}	118 ± 12^{c*}	158 ± 21^{a*}	181 ± 26^{b*}	213 ± 25^{b*}	291 ± 55^{b*}
	10N		36.1 ± 1.1^{a*}	49.9 ± 1.9^{b*}	61.9 ± 4.4^{c*}	92.4 ± 2.8^{c*}	138 ± 8^{d*}	184 ± 11^{b*}	244 ± 13^{c*}	269 ± 21^{c*}	284 ± 17^{b*}
N	5A	6.4 ± 0.2	7.4 ± 0.3^{a*}	9.5 ± 0.5^{a*}	10.8 ± 0.3^{a*}	17.1 ± 0.7^{a*}	32.1 ± 2.4^{a*}	35.8 ± 3.3^{a*}	38 ± 5.1^{a*}	50.0 ± 5.1^{a*}	51.4 ± 6.0^{a*}
	5N		7.5 ± 0.1^{a*}	9.3 ± 0.7^{a*}	13.4 ± 0.6^{b*}	19.0 ± 1.0^{b*}	27.2 ± 1.2^{b*}	31.4 ± 4.0^{b*}	45.7 ± 3^{b*}	54.0 ± 4.2^{b*}	64.3 ± 6.7^{b*}
	10A		7.7 ± 0.1^{a*}	9.9 ± 0.5^{b*}	11.6 ± 1.4^{c*}	15.4 ± 3.0^{a*}	29.9 ± 3.0^{c*}	39.0 ± 4.2^{c*}	46.2 ± 5.2^{b*}	53.0 ± 5.7^{b*}	70.0 ± 11^{b*}
	10N		7.6 ± 0.2^{a*}	10.5 ± 0.3^{b*}	14.5 ± 0.8^{d*}	22.7 ± 2.0^{c*}	33.4 ± 1.4^{d*}	44.0 ± 1.7^{d*}	59.5 ± 3.5^{c*}	64.2 ± 3.3^{c*}	68.5 ± 4.2^{b*}
Lipid	5A	7.7 ± 1.1	8.5 ± 0.2^{a}	8.7 ± 0.2^{a}	8.8 ± 0.2^{a}	9.3 ± 0.2^{a*}	16.4 ± 0.6^{a*}	28.6 ± 0.3^{a*}	39.3 ± 0.6^{a*}	52.6 ± 0.2^{a*}	63.1 ± 0.6^{a*}
	5N		8.3 ± 0.3^{a}	8.8 ± 0.4^{a}	8.6 ± 0.2^{a}	9.8 ± 0.1^{b*}	16.3 ± 0.4^{a*}	27.6 ± 0.1^{b*}	39.5 ± 0.6^{a*}	51.7 ± 0.3^{b*}	62.7 ± 0.8^{a*}
	10A		8.3 ± 0.3^{a}	9.0 ± 0.2^{a}	9.1 ± 0.1^{b}	9.9 ± 0.2^{b*}	18.0 ± 0.3^{b*}	28.7 ± 0.3^{a*}	39.2 ± 0.2^{a*}	51.7 ± 0.3^{b*}	63.6 ± 0.5^{b*}
	10N		8.2 ± 0.2^{b}	9.0 ± 0.3^{a}	8.6 ± 0.2^{a}	9.8 ± 0.1^{b*}	17.7 ± 0.2^{b*}	27.5 ± 0.7^{b*}	39.7 ± 0.4^{a*}	52.2 ± 0.3^{b*}	63.3 ± 0.2^{b*}
Protein	5A	20.6 ± 2.5	26.8 ± 1.0^{a}	27.5 ± 0.4^{a}	31.2 ± 0.4^{a*}	35.2 ± 0.1^{a*}	80.1 ± 2.8^{a*}	109 ± 2^{a*}	146 ± 1^{a*}	220 ± 1^{a*}	254 ± 1^{a*}
	5N		26.2 ± 0.4^{a}	26.8 ± 0.6^{a}	30.6 ± 0.6^{a*}	35.4 ± 0.4^{b*}	80.9 ± 0.5^{a*}	109 ± 2^{a*}	144 ± 1^{b*}	220 ± 1^{a*}	255 ± 2^{a*}
	10A		27.0 ± 0.3^{a}	27.4 ± 0.5^{a}	33.3 ± 0.4^{b*}	35.0 ± 0.5^{b*}	82.9 ± 1.3^{b*}	109 ± 2^{a*}	147 ± 1^{c*}	220 ± 2^{a*}	254 ± 1^{a*}
	10N		27.2 ± 0.5^{a}	27.0 ± 0.8^{a}	32.8 ± 0.8^{b*}	36.0 ± 0.5^{b*}	80.1 ± 1.2^{b*}	110 ± 1^{a*}	147 ± 1^{c*}	220 ± 2^{a*}	254 ± 1^{a*}

Table 2. *Macrobrachium amazonicum*, comparison of body size (measured as carapace length, CL) of first-stage juveniles (instar 10) after larval rearing at two different salinities (5, 10) and with two different types of salt (A: artificial, N: natural); mean values ± SD; % = relative CL, compared with the optimum condition (treatment 10N: CL = 100%); n: number of individuals measured, different lower case letters indicate significant differences between treatments (SNK tests); ANOVA, $F_{3,132}$ = 10.411; P < 0.05.

Treatment	CL		
	mm	%	n
5A	2.11 ± 0.10^{a}	81	43
5N	2.30 ± 0.14^{b}	89	28
10A	2.40 ± 0.13^{c}	92	24
10N	2.61 ± 0.12^{d}	100	41

The percentage values of lipid and protein showed no consistent response to different salinities or types of salt. The lipid content increased slightly, from initially about 14% to ca. 16-19% of W in larval zoea VI, remaining stable thereafter. Maximum values were observed at salinity 10, regardless of natural or artificial salt. A minimum lipid value (11% of W) was measured in early juveniles (Table 3). The percentage of protein increased from about 36% after hatching to >50% in later larval stages. A maximum protein level was found in first-stage juveniles.

Statistical analysis of developmental and treatment effects on larval growth and chemical composition of biomass

An overall comparison of the effects of the experimental factors "Salinity" (5 *vs.* 10), "Type of salt" (artificial *vs.* natural), "Female", and "Stage", is compiled in Table 4. Four-way ANOVA (nested design) revealed significant effects of all four factors on all biomass parameters, although at different levels of significance. Interactions between these factors were in all cases statistically significant. The developmental "Stage" had consistently the strongest influence on all biomass parameters per individual and on the percentage values of protein, C, N, and lipid, as well as the C/N ratio (all P < 0.001). The factor "Salinity" had generally stronger effects on biomass per individual and chemical composition (all P < 0.01) than the factor "Type of salt" (all P < 0.05).

DISCUSSION

M. amazonicum most probably represents a complex of closely related species comprising estuarine clades in

Table 3. *Macrobrachium amazonicum*, changes in elemental and proximate biochemical composition of larval biomass (contents of C, N, lipid, protein; all in % of W); C/N mass ratio; for further explanation, see Table 1.

Biomass (%, ratio)	Exp.	Stage									
		I	II	III	IV	V	VI	VII	VIII	IX	X
C	5A	54.3 ± 0.6	49.1 ± 0.5^{a*}	48.1 ± 0.8^{a}	44.1 ± 0.8^{a*}	44.1 ± 0.5^{a}	44.0 ± 0.6^{a}	43.2 ± 0.7^{a*}	44.4 ± 0.9^{a}	42.4 ± 0.6^{a*}	42.6 ± 0.8^{a}
	5N		49.1 ± 0.3^{a*}	48.6 ± 2.7^{a}	44.1 ± 0.5^{a*}	44.1 ± 0.2^{a}	43.0 ± 0.6^{b}	44.1 ± 2.4^{a*}	44.8 ± 0.5^{a}	46.3 ± 0.5^{b*}	45.0 ± 0.8^{b}
	10A		49.0 ± 0.1^{a*}	45.8 ± 1.2^{b}	43.9 ± 0.1^{a*}	41.6 ± 0.5^{b*}	42.4 ± 0.8^{b}	42.4 ± 0.5^{a*}	43.0 ± 1.1^{b}	44.2 ± 1.2^{c*}	45.5 ± 1.2^{b}
	10N		48.4 ± 0.5^{a*}	46.6 ± 0.5^{b}	44.8 ± 0.5^{b*}	43.3 ± 0.5^{c*}	43.5 ± 0.7^{c}	41.1 ± 3.8^{a}	45.6 ± 0.6^{c}	46.5 ± 2.1^{b*}	46 ± 1.02^{b}
N	5A	10.1 ± 0.1	10.5 ± 0.1^{a}	10.3 ± 0.1^{a*}	11.1 ± 0.2^{a}	11.3 ± 0.1^{a}	10.7 ± 0.1^{a*}	10.7 ± 0.1^{a}	11.6 ± 0.1^{a*}	10.8 ± 0.05^{a}	10.8 ± 0.2^{a}
	5N		10.4 ± 0.1^{b}	10.5 ± 0.6^{a*}	10.5 ± 0.1^{b}	11.1 ± 0.1^{b}	10.6 ± 0.1^{a*}	10.8 ± 0.9^{a}	11.0 ± 0.2^{b*}	10.7 ± 0.1^{a}	10.5 ± 0.1^{b}
	10A		10.2 ± 0.1^{c}	9.6 ± 0.3^{b*}	11.3 ± 0.1^{c}	11.3 ± 0.1^{a}	10.7 ± 0.3^{a*}	10.4 ± 0.2^{b}	10.9 ± 0.2^{b}	10.9 ± 0.1^{a}	10.9 ± 0.2^{a}
	10N		10.2 ± 0.1^{c}	9.8 ± 0.1^{b*}	10.5 ± 0.1^{b}	10.6 ± 0.1^{c}	10.4 ± 0.1^{b}	9.8 ± 0.4^{c}	11.0 ± 0.1^{b*}	11.0 ± 0.2^{b}	11.0 ± 0.3^{a}
C/N	5A	5.33 ± 0.13	4.64 ± 0.03^{a*}	4.62 ± 0.04^{a}	4.01 ± 0.07^{a*}	3.90 ± 0.07^{a*}	4.07 ± 0.06^{a}	4.03 ± 0.09^{a}	3.81 ± 0.06^{a*}	3.88 ± 0.05^{a}	3.93 ± 0.11^{a}
	5N		4.68 ± 0.04^{b*}	4.62 ± 0.03^{a}	4.17 ± 0.04^{b}	3.97 ± 0.03^{b*}	4.03 ± 0.09^{a}	4.07 ± 0.15^{b}	4.04 ± 0.09^{b}	4.23 ± 0.06^{b}	4.25 ± 0.07^{b}
	10A		4.74 ± 0.02^{c*}	4.77 ± 0.02^{b}	3.87 ± 0.05^{c}	3.67 ± 0.07^{c*}	3.96 ± 0.09^{b}	4.07 ± 0.11^{b}	3.92 ± 0.17^{b*}	4.04 ± 0.14^{c}	4.15 ± 0.15^{b}
	10N		4.73 ± 0.06^{c*}	4.74 ± 0.04^{b}	4.26 ± 0.08^{d}	4.06 ± 0.09^{d*}	4.15 ± 0.06^{c}	4.19 ± 0.09^{c}	4.12 ± 0.09^{b}	4.2 ± 0.14^{b}	4.16 ± 0.04^{c}
Lipid	5A	13.8 ± 0.2	13.6 ± 0.2^{a}	13.9 ± 0.4^{a}	14.1 ± 0.3^{a}	14.9 ± 0.2^{a*}	16.5 ± 1.0^{a*}	16.1 ± 0.5^{a}	13.2 ± 0.9^{a*}	14.7 ± 0.4^{a*}	11.3 ± 1.0^{a*}
	5N		13.3 ± 0.5^{a}	14.1 ± 0.6^{b}	13.7 ± 0.3^{b}	15.6 ± 0.2^{b*}	16.3 ± 0.6^{a*}	14.4 ± 0.9^{b}	13.6 ± 1.0^{a*}	13.2 ± 0.5^{b}	10.9 ± 1.3^{a*}
	10A		13.4 ± 0.5^{a}	14.4 ± 0.4^{b}	14.6 ± 0.1^{c}	16.0 ± 0.3^{c*}	19.1 ± 0.5^{b*}	16.1 ± 0.5^{a}	13.0 ± 0.4^{b*}	13.2 ± 0.5^{b}	12.4 ± 0.9^{b*}
	10N		13.1 ± 0.3^{b}	14.4 ± 0.5^{b*}	13.8 ± 0.2^{b}	15.8 ± 0.2^{c*}	18.5 ± 0.4^{b*}	14.3 ± 1.2^{b}	13.9 ± 0.7^{c*}	14.1 ± 0.5^{c}	11.8 ± 0.3^{c*}
Protein	5A	36.2±1.9	43.1 ± 1.7^{a*}	44.2 ± 0.6^{a}	50.2 ± 0.7^{a*}	56.6 ± 0.2^{a*}	53.9 ± 4.5^{a*}	55.5 ± 1.2^{a}	55.4 ± 2.3^{a*}	54.9 ± 2.4^{a}	59.0 ± 1.7^{a*}
	5N		42.2 ± 0.6^{b*}	43.1 ± 0.9^{b}	49.2 ± 0.9^{b*}	57.0 ± 0.6^{b*}	55.2 ± 0.9^{a*}	55.3 ± 3.2^{a}	52.6 ± 3.3^{b}	54.7 ± 2.7^{a}	60.7 ± 2.9^{b*}
	10A		43.5 ± 0.5^{c*}	44.1 ± 0.8^{c}	53.6 ± 0.9^{c*}	56.2 ± 0.5^{c*}	58.4 ± 2.2^{b*}	55.5 ± 3.1^{a}	56.7 ± 0.3^{c}	54.1 ± 2.7^{a}	60.0 ± 0.6^{c*}
	10N		43.7 ± 0.8^{c*}	43.3 ± 1.3^{c}	53.0 ± 1.2^{c*}	57.2 ± 0.8^{d*}	53.8 ± 2.0^{a*}	57.7 ± 2.6^{b}	56.7 ± 2.2^{c*}	54.5 ± 2.4^{a}	58.7 ± 0.8^{d*}

northern and northeastern South America as well as distinct hololimnetic clades in the hydrologically separated La Plata Basin and, possibly, in central Amazonia (Vergamini *et al.*, 2011; dos Santos *et al.*, 2013; Weiss *et al.*, 2015). Although biologically relevant differences between separate populations have been found in reproduction, ecology, morphology, growth, larval development, physiology, and genetics (Dos Santos *et al.*, 2013; Hayd & Anger, 2013; Weiss *et al.*, 2015; Boudour-Boucheker *et al.*, 2016), there remains also a conflicting hypothesis proposing a single species with an enormous geographic distribution range (>4.000 km across) and an extreme degree of phenotypic plasticity, *i.e.*, environmentally induced changes in biological traits (Vergamini *et al.*, 2011; Maciel *et al.*, 2014). A recent study (Soeiro *et al.*, 2016) showed, however, that the early larval stages (I-IV) of two different populations of *M. amazonicum* (both from the lower Amazon region; one living in estuarine habitats with salinities >25, another one from a site characterized by fresh water), did not show any significant effect of the habitats of origin on salinity tolerance. These data indicate that the degree of phenotypic plasticity of this species is rather limited, which supports the interpretation of differential biological traits as indicators of genetic diversification.

Since the present study deals with a population originating from a place close to the type locality of the species *M. amazonicum* (originally described by Heller (1862), as *Palaemon amazonicus*) in northern Pará, our results may be considered as representative for the salinity requirements of the larvae of this species from the lower Amazon. However, closely related but separate species from the La Plata Basin and, possibly, from the central and upper Amazon regions may differ in their response to different salinities or types of salt, as their capabilities of ionic and osmotic regulation are much stronger (Charmantier & Anger, 2011; Boudour-Boucheker *et al.*, 2016).

M. amazonicum, which is commonly referred to as "Amazon River prawn", can grow to large body size (up to about 16 cm total length or 30 g fresh weight) and is therefore considered as a candidate for commercial aquaculture (New, 2005; Moraes-Valenti & Valenti, 2010; Hayd *et al.*, 2010, 2014). Optimum physical conditions for larval rearing have been found at salinities 10-15 (Guest & Durocher, 1979; Barreto & Soares, 1982; Araujo & Valenti, 2007). Our experiments show, however, that successful development from hatching to the first juvenile stage is possible also at salinity 5. The larvae are, compared to conspecific adults, weak hyper-osmoregulators (Charmantier & Anger, 2011; Boudour-Boucheker *et al.*, 2016). However, moderate osmotic stress does not severely affect larval survival, and it causes only slightly reduced growth. This effect of a suboptimal salinity has been quantified in the present study. It reveals significantly weaker gains in larval dry weight (W) and organic biomass (C, N, lipid, protein) per larva as well as smaller body size of first-stage juveniles at salinity 5. The relative chemical composition of W (elemental and major biochemical constituents in % of W; C/N

Table 4. *Macrobrachium amazonicum*, statistical analysis of larval growth (biomass per individual, see Table 1) and changes in elemental and biochemical composition of biomass (% of W, C/N mass ratio; see Table 3); 4-way ANOVA (nested design), with salinity and type of salt as independent factors; female and developmental stage as nested factors; effects of four experimental treatments (Salinity, Type of salt, Female, Stage), and interactions between these factors on biomass parameters; df: degrees of freedom, MS: mean square, F: F-value, *P*: significance level; further explanations: see Tables 1, 3.

Biomass parameter	Factor, Interactions	df	MS	F	*P*
W (µg ind^{-1})	Salinity	1	20273	19.5	<0.01
	Type of salt	1	13821	13.3	<0.05
	Type of salt×salinity	2	10286	9.87	<0.05
	Female (type of salt×salinity)	16	63031	60.5	<0.05
	Stage (Female (type of salt×salinity))	160	18731	18	<0.001
	Error	180	1042.03		
C (µg ind^{-1})	Salinity	1	7051	24.1	<0.01
	Type of salt	1	5283	18.1	<0.05
	Type of salt×salinity	2	2028	6.95	<0.05
	Female (type of salt×salinity)	16	124389	426	<0.05
	Stage (Female (type of salt×salinity))	160	22431	76.8	<0.001
	Error	180	292		
N (µg ind^{-1})	Salinity	1	321	29.4	<0.01
	Type of salt	1	185	17	<0.05
	Type of salt×salinity	2	95.7	8.78	<0.05
	Female (type of salt×salinity)	16	1644	151	<0.05
	Stage (Female (type of salt×salinity))	160	529	48.5	<0.001
	Error	180	10.9		
Lipid (µg ind^{-1})	Salinity	1	10.9	57.4	<0.01
	Type of salt	1	6.26	32.9	<0.05
	Type of salt×salinity	2	2.17	11.4	<0.05
	Female (type of salt×salinity)	16	243	1279	<0.05
	Stage (Female (type of salt×salinity))	160	31.5	166	<0.001
	Error	180	0.19		
Protein (µg ind^{-1})	Salinity	1	76.1	56.4	<0.01
	Type of salt	1	51.3	38	<0.05
	Type of salt×salinity	2	22.4	16.6	<0.05
	Female (type of salt×salinity)	16	442	327	<0.05
	Stage (Female (type of salt×salinity))	160	121	89.6	<0.001
	Error	180	1.35		
C (% W)	Salinity	1	41.9	31.3	<0.01
	Type of salt	1	28.7	21.4	<0.05
	Type of salt×salinity	2	11.3	8.43	<0.05
	Female (type of salt×salinity)	16	92.7	69.2	<0.05
	Stage (Female(type of salt×salinity))	160	22.1	16.5	<0.001
	Error	180	1.34		
N (%W)	Salinity	1	1.09	13.6	<0.01
	Type of salt	1	0.88	11	<0.05
	Type of salt×salinity	2	0.64	8	<0.05
	Female (type of salt×salinity)	16	3.32	41.5	<0.05
	Stage (Female (type of salt×salinity))	160	1.47	18.4	<0.001
	Error	180	0.08		
C/N mass ratio	Salinity	1	1.04	52	<0.01
	Type of salt	1	0.81	40.5	<0.05
	Type of salt×salinity	2	0.22	11	<0.05
	Female (type of salt×salinity)	16	2.49	124.5	<0.05
	Stage (Female (type of salt×salinity))	160	1.65	82.5	<0.001
	Error	180	0.02		
Lipid (% W)	Salinity	1	6.43	16.1	<0.01

Continuation

Biomass parameter	Factor, Interactions	df	MS	F	*P*
	Type of salt	1	4.92	12.3	<0.05
	Type of salt×salinity	2	3.21	8.03	<0.05
	Female (type of salt×salinity)	16	40.1	100	<0.05
	Stage (Female (type of salt×salinity))	160	9.27	23.2	<0.001
	Error	180	0.4		
Protein (% W)	Salinity	1	111	20.7	<0.01
	Type of salt	1	72.7	13.6	<0.05
	Type of salt×salinity	2	3.21	8.03	<0.05
	Female (type of salt×salinity)	16	543	101	<0.05
	Stage (Female (type of salt×salinity))	160	121	22.6	<0.001
	Error	180	5.35		

mass ratio), by contrast, showed less clear and less consistent response patterns than the biomass per individual. It remains to be elucidated in future studies whether these effects are caused indirectly, by a reduction of larval feeding activity under suboptimal rearing conditions, or directly, affecting the physiological conversion of ingested food into body mass.

Our experiments revealed that the cultivation of this species does not necessarily require natural sea salts, but can be achieved also with artificial salts. Again, however, this rearing condition deviates from the optimum, causing reduced larval growth and smaller juvenile size. These effects were more pronounced at the lower salinity (5 *vs.* 10), especially in organic biomass per individual, probably due to an unfavorable ionic composition of artificial salts (*e.g.*, low K^+ concentration; Roy *et al.*, 2007).

Reduced larval biomass accumulation was reflected in smaller body size of early juveniles. This shows carry-over effects of variation in larval fitness persisting from one phase of the life cycle to the next (for a recent discussion of such "trait-mediated effects", see Giménez, 2004, 2010). Although being statistically significant, however, the negative effects of slightly suboptimal rearing conditions (low salinity and/or use of artificial salt) on larval growth and early juvenile size were generally weak. Among the rearing conditions tested in this study, maximum reduction of juvenile body size was 19% in the pessimum treatment (5A) as compared to the optimum (10N; see Table 2). In aquaculture, these effects may be considered as tolerable trade-offs which they can be compensated for by a reduction of the costs and of logistic problems that are associated with cultivation systems requiring natural seawater. This weighting should be particularly relevant for cultivation facilities located at large distance from the coast, where seawater transport is not feasible. However, it remains to be tested as to which extent such changes in larval quality and first-stage postlarval size may propagate through subsequent juvenile stages, which eventually might affect the final adult body size, marketing quality, or the time to reproduction. Also, different conditions and techniques of larval rearing used in different laboratories or aquaculture facilities must be taken into account when data of larval growth and development are compared in the literature.

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