www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 17: 767-775 (2017) DOI: 10.4194/1303-2712-v17_4_12

RESEARCH PAPER

Population Growth and Protein and Energy Content of *Proales Similis* (Rotifera: Monogononta) Reared at Different Salinities

José Cristóbal Román Reyes[1], Cynthia Jazmín López Monteón[2], Hipólito Castillo Urreta[3], María del Carmen Monroy Dosta[4], Gustavo Alejandro Rodríguez Montes de Oca[1,*]

[1]Laboratorio de reproducción y cultivo de peces, Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Mazatlán, México.
[2]Instituto Tecnológico de Tepic. Tepic, Nayarit, México.
[3]Escuela de Biología, Universidad Autónoma de Sinaloa, Culiacán Rosales, Sinaloa, México.
[4]Universidad Autónoma Metropolitana-Unidad Xochimilco. Depto. El Hombre y su Ambiente. Coyoacán, México.

* Corresponding Author: Tel.: +52.669 1814586;
E-mail: grodriguez@uas.edu.mx

Received 05 October 2016
Accepted 10 January 2017

Abstract

The effect of different salinities on growth, crude protein and total energy on a Mexican strain of the minute rotifer *Proales similis* (GenBank KM078762) were evaluated. The experiment was conducted at 5, 15, 25 and 35 psu, with five replicates. Rotifers were fed twice a day with *Nannochloropsis* sp., temperature, dissolved oxygen and pH values were maintained at 25±1 °C, 5±1 mg L^{-1} and 8±1 respectively. Results show that salinity does not affect the intrinsic rate of population growth (r=0.46 to 0.51 day^{-1}) and duplication time (Dt=1.36 to 1.51 days) for *P. similis*. Maximum density (Dmax) values were lower at 35 psu (1,703 rotifers mL^{-1}), whereas Dmax values were between 2,488 to 2,560 rotifers mL^{-1} at 5, 15 and 25 psu. Crude protein fluctuated between 25.3 and 42.8% dry biomass (DB), while energy content varied between 11.8 and 19.9 J mg^{-1} DB; values were significantly lower at 35 psu. Observed population growth parameters and the protein and energy content between 5-25 psu, indicate that *P. similis* can be successfully reared below 35 psu, making *P. similis* very suitable when fish larvae require a small rotifer due to sizing of mouth gap at the beginning of exogenous feeding.

Keywords: Energy, *Proales*, protein, rotifer, salinity.

Introduction

One of the major challenges facing modern aquaculture is to achieve diversification and controlled culture of different fish species in order to meet the demand for animal protein in human nutrition and to restore populations of threatened or endangered species (Braithwaite & Salvanes, 2010). However, a problem that affects the production of marine species, is that most larvae hatch with little yolk reserves that are consumed in a short time. In some species size and the opening of their mouths are very small, their digestive system is primitive and their perceptual abilities for finding and catching food are also poor. This stage, in which the larva requires exogenous feeding, is a critical period of high mortality that poses serious challenges for marine aquaculture (Takeuchi, 2014). Rotifers of the *Brachionus plicatilis* species complex are the first live feed for many marine fish larvae; among these, *Brachionus rotundiformis* is the preferred rotifer for the initial feeding of larvae with small mouths, due to its smaller size (lorica length 100-210 µm, mean±SD: 148.7±1.3 µm) (Ciros-Pérez, Gómez, & Serra, 2001). Unfortunately, the early larval stages of several species do not consume these rotifers because their mouth openings are extremely small (<130 µm). This group includes snappers of the Lutjanidae family (Schipp, Bosmans, & Marshall, 1999), snooks of the Centropomidae family, combers and groupers of the Serranidae family (Toledo, Golez, Doi, & Ohno, 1999), and Pacific fat sleepers or *Dormitator latifrons* of the family Eleotridae (Rodríguez Montes de Oca, Medina-Hernández, Velázquez-Sandoval, López-López, Román-Reyes, Dabrowski, & Haws, 2012). The larvae of these groups of fish require small live feed of between 40 and 80 µm in their early feeding stages (Olivotto, Holt, Carnevali, & Holt, 2006); consequently, there is a growing demand in the aquaculture industry for very small live feed to be used in the first feeding of marine fish larvae with extremely small mouths.

The following alternatives have been tested in the search for very small live feed: protozoa, diatoms and dinoflagellates (Nagano, Iwatzuki, KAmiyama, Shimzu, & Nakata, 2000; Nakagawa, Senoo, & Miyashita, 2007; Das, Mandal, Bhagabati, Akhtar, & Singh, 2014), rotifers of the genera *Colurella* and *Lecane* (Chigbu & Suchar 2006; Lahope, Wullur, Rimper, Pangkey, & Rumengan, 2013), bivalve larvae, sea urchin eggs, nauplii of balanus, trochophore larvae of *Crassostrea* oysters, and nauplii

of copepods (Rimmer 2000; Treece & Davis, 2000); however, the results have not been satisfactory because some of these foods have low nutritional value or are very difficult to obtain in high densities.

There have been recent studies on the management and production of rotifers belonging to other taxonomic genera, notably a series of papers presented by Wullur, Sakakura, and Hagiwara (2009); Wullur, Sakakura, and Hagiwara (2011); Hirai, Koiso, Teruya, Kobayashi, Takebe, Sato, Nakamura, Goto, and Hagiwara (2012); Wullur, Yoshimatsu, Tanaka, Ohtani, Sakakura, Kim, and Hagiwara (2013); Hagiwara, Wullur, Marcial, Hirai, and Sakakura (2014) and Tomoda, Furuita, Kamoshida, Kurogi, Shibuno, Tanaka, and Tezuka (2014), in which they describe some biological and nutritional characteristics of the rotifer *Proales similis*, which was isolated from an estuary in the Ishigaki island, Okinawa, Japan, and was used successful in the first feeding of larval fish with extremely small mouths. They described this species as a small size (80±3.4 µm), soft-bodied (aloricated) rotifer that reaches high densities in laboratory cultures. This rotifer is euryhaline, consumes live microalgae and commercial products and is also cosmopolitan. This rotifer can be cultured under different salinities, with good population growth (Wullur *et al.* 2009), which is highly relevant when considering the different salt conditions where some aquaculturally important fish larvae are reported to grow and survive (Wang, Li, Cui, & Lu, 2013).

Given this, we recently isolated and morphologically identified a native strain of the species *P. similis* from eggs with diapause embryos deposited in shrimp pond sediments of a farm located south of Mazatlan, Sinaloa, Mexico. No basic studies have been conducted on this strain to explore its potential as live feed.

Population growth studies outline the development of the life cycle, fertility and hatchability of eggs and are considered as the most direct way to assess rotifer productivity (Malekzadeh-Viayeh, Mohammadi, & Shafiei, 2010); moreover, they are especially useful because the growth potential of rotifers can have strain-specific features, and such studies provide valuable information of native species recently identified (Miracle & Serra, 1989; Malekzadeh-Viayeh, 2012). The criteria for selecting live feed include adaptability to simple techniques of mass production and tolerance to wide ranges of environmental factors without significantly altering nutritional quality (Das, Mandal, Bhagabati, Akhtar, & Singh, 2012). It has been found that even in euryhaline organisms; salinity influences the growth and biochemical quality of rotifers, because salinity stress involves an energy expenditure which is diverted into osmoregulation instead of being allocated to somatic growth and reproduction (Lowe, Kemp, Bates, & Montagnes., 2005). With the above assumptions, this paper investigates the effect of salinity on the growth and protein and energy content of *P. similis* in order to characterize the species and explore its potential use as live feed for larval feeding.

Materials and Methods

Experimental Organisms

The rotifer used in this study was isolated after the hatching of diapause eggs naturally present in the sediment of white shrimp (*Litopenaeus vannamei*) production ponds of a local farm in southern Sinaloa, Mexico (23° 9' 10.54" N, 106° 18' 22.84" W) using the procedure described by Román-Reyes, Castañeda-Rodríguez, Castillo-Ureta, Bojórquez-Domínguez, and Rodriguez-Montes de Oca, (2014). A native strain of the rotifer *P. similis* (length 90±1.94 µm, width 56±1.66 µm) was identified, based on their external morphological characteristics and mastax, using the keys of Koste (1978) and Segers (1995). Subsequently, to confirm the identity of the species, we carried out a molecular analysis, sequencing a fragment of the gene cytochrome oxidase I (COI) from mitochondrial DNA; using the BLAST® program, we aligned those sequences with sequences of the taxon Rotifera and identified the species *P. similis*. The obtained sequence was deposited in the GenBank® database with the access code KM078762. The strain is available at the laboratory of fish reproduction and culture of the Faculty of Marine Sciences at the Autonomous University of Sinaloa, Mazatlan, Sinaloa, Mexico.

Experimental Design

The experiments were performed at salinities of 5, 15, 25 and 35 practical salinity units (psu), using transparent bottles (12 L) for a total of 10 days with five replicates per salinity. In all experiments, the temperature was maintained at 25±1° C in a controlled temperature room. The seawater pumped from the Mazatlan Bay (between 34 and 36 psu), previously treated with a serial filter system with cartridges of 20, 10, 5 and 1 microns and finally sterilized with UV filters. The freshwater water used to prepare salinities <35 psu was obtained from the drinking water network and treated according to the procedure described for seawater.

Before starting the experiments, the rotifers were acclimated to the experimental salinities for at least one week and the density of rotifers was subsequently adjusted to 50 rotifers mL^{-1} in each of the replicates for all treatments. The rotifers were fed twice a day by the microalgae *Nannochloropsis* sp. at a concentration of $3x10^6$ cells ml^{-1}, at intervals of 6 to 12 h. The microalgae were grown in filtered sea water, disinfected with sodium hypochlorite and neutralized with sodium thiosulphate; F medium of Guillard and Ryther (1962) was used as culture media. During the experiments, air diffusers were used to maintain the

dissolved oxygen concentration above 4 mg L^{-1}, while the pH was maintained at 8.0±1.0 using drops of a solution of commercial hydrochloric acid (1-M HCl). Temperature and dissolved oxygen were monitored daily using a dissolved oxygen meter YSI 550A, while pH was measured with a potentiometer Hanna HI 98128W, new water was added to experimental units with each microalgae addition.

Determination of Rotifer Population Dynamics (Intrinsic Rate of Population Growth, Duplication Time and Maximum Density)

The density of rotifers was determined based on the average of triplicate samples of 1 mL, which were collected from the culture unit with a pipette and then placed in a Sedgwick-Rafter chamber for counting zooplankton. The organisms were fixed with a Lugol solution and then counted under a stereoscopic microscope. The intrinsic rate of population growth (r) was calculated using the equation suggested by Øie *et al*. (1994): r=(lnNt-lnNo)/t, where No is the initial concentration of experimental organisms, Nt is the concentration of individuals at time t and t is the culture period in days. The duplication time (Dt) of the population was determined by the function Dt=ln2/r, while the maximum density (Dmax) was estimated using the maximum abundance reached after 10 days of culture; Tmax is the time it took the population to reach Dmax.

Determination of Dry Weight and the Organic Content of Rotifers

To determine the dry and organic weight of rotifers, we collected samples of 100 mL with a density of 300 rotifers mL^{-1} from each replicate; these were then concentrated using fiberglass filters Whatman GF/C of 25 mm in diameter previously calibrated in a semi-micro analytical scale (Denver Instrument) with a precision of 0.01 mg. The residues of marine salts were removed with an aqueous solution of ammonium formate during the concentration of the samples (4%). Afterwards, the samples were dried in successive periods of 24 h in a convection oven at 60°C until a constant weight was achieved in the semi-micro analytical balance. Finally, the samples were incinerated in a muffle furnace at 450°C for at least 12 h while still monitoring for constant weight. The value thus obtained represented the inorganic ash content of the samples; the organic biomass content was calculated as the difference between total dry weight and the weight of the ash content.

Determination of Crude Protein and Energy

When the maximum density of rotifers in the cultures was reached, we collected samples of 500 mL from each replicate; these were then washed and the rotifers were retained on a sieve of 20 µm and resuspended for 30 minutes in clean filtered water at the same salinity to allow egestion of ingested food. After counting them, the rotifers were then concentrated, placed in 5 ml vials, immediately stored in a deep freezer (-70°C) and lyophilized shortly thereafter at -46°C/-50°C and 55 MBR of pressure during 24 hours. The samples were then homogenized in agate mortars. One mg of each sample was weighed in an analytical balance (Denver Instrument) with an accuracy of 0.01 mg, packed in tin capsules of 4x6 mm (Costech Inc., Valencia CA) and stored in plastic boxes of 96 wells with bidimensional identification. The samples were sent for analysis to the stable isotope laboratory of the University of California, Davis, USA. The nitrogen (N) and carbon (C) content of the samples was determined using an elemental analyzer C-N (PDZ Europa, ANCA-GSL). Crude proteins were determined by multiplying the nitrogen content (%N) by the conversion factor of 4.46 for rotifers (Srivastava *et al*., 2006), while the energy content was obtained by multiplying the carbon content by the factor of 10.9 cal mg^{-1} C (Salonen, Sarvala, Hakala, & Viljanen, 1976), and was subsequently converted to J mg^{-1} of dry biomass (DB).

Statistical Analysis

The effects of salinity on the variables of population dynamics, biomass, elemental composition and protein and energy content of the rotifers were evaluated using one-factor analysis of variance (ANOVA). Previously, the data were subjected to the Lilliefors normality test and to Bartlett's variance homogeneity test (Zar, 1999). Tukey multiple comparisons tests were applied when the results of ANOVA were significant ($P<0.05$).

Results

Population Growth

The growth of *P. similis* at different salinities used showed that the population increased very slowly during the first four days of culture, and that the exponential growth phase started on the fifth day, reaching maximum density (Dmax) at a time (Tmax) of eight days of culture (Figure 1). The parameters of the growth curves indicated that salinity did not significantly affect the intrinsic growth rate (r) (g.l.=16, F=2.06, P=0.18) nor the duplication time (Dt) (g.l.=16, F=2.20, P=0.17). The growth rate (r) varied between 0.46 and 0.52 day^{-1} at the experimental salinities; and the Dt were between 1.36 and 1.51 days (Table 1). The tests showed a significant effect of salinity on Dmax (g.l.=16, F=43.77, P<0.001); the density of *P. similis* was highest when grown at 5, 15 and 25 psu, with very similar average maximum densities of 2546, 2560 and

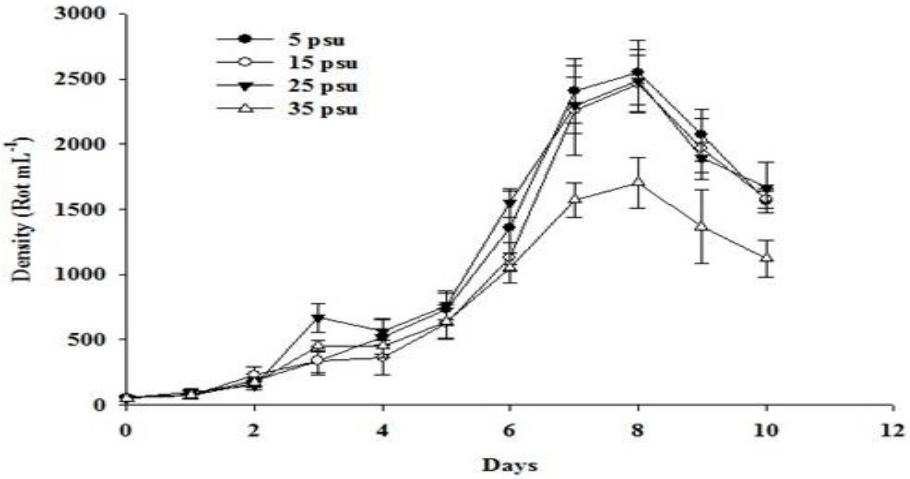

Figure 1. Population growth curves for *P. similis* reared at different salinities. Symbols indicate mean density ± SD for a 10 day culture period. Some SD are not distinguishable due to small values.

Table 1. Intrinsic rate of population growth (*r*), duplication time (Dt), maximum density (Dmax) and time for maximum density (Tmax) for *Proales similis* reared at different salinities (Mean ± SD). Different letters indicate significant differences (P<0.05) within columns

	Population growth parameters			
Salinity (psu)	*r* (day^{-1})	Dt (days)	Dmax (rot mL^{-1})	Tmax (days)
5	0.51±0.10^{a}	1.36±0.09^{a}	2546±105^{b}	8
15	0.50±0.08^{a}	1.39±0.09^{a}	2560±185^{b}	8
25	0.50±0.09^{a}	1.39±0.08^{a}	2488±135^{b}	8
35	0.46±0.07^{a}	1.51±0.10^{a}	1703±130^{a}	8

2488 rot mL^{-1}, respectively, and a Tmax of eight days, from an initial density of 50 rot mL^{-1}. Rotifers grown at 35 psu were next, with a significantly lower Dmax of 1703 rot mL^{-1} and a Tmax of 8 days (Table 1).

Biomass and Elemental Composition

In general, rotifers showed a mean total length±standard deviation of 90±1.94 µm (minimum: 85, maximum: 94 µm) and body width of 56±1.66 µm (minimum: 23, maximum: 64 µm). Figure 2 shows the results of dry biomass, organic biomass and ash content of rotifers in saline treatments. Dry biomass increased significantly (g.l.=16, F=66.57, P<0.001) with increasing salinity, with values of 13.2, 25.3 and 39.9 ng rot^{-1} at salinities of 5, 15 and 25 psu, respectively; it then decreased to 32.4 ng rot^{-1} at a salinity of 35 psu. Organic biomass (OB) presented a similar pattern to that recorded for dry biomass; it increased significantly (g.l.=16, F=32.35, P<0.001) from salinities of 5 (7.2 ng rot^{-1}), 15 (19.3 ng rot^{-1}) and 25 (27.5 ng rot^{-1}) psu, and then decreased to 18.2 ng rot^{-1} with 35 psu, similar to that recorded in 15 psu. Rotifer ash content (AC) differences (g.l.=16, F=66.16, P<0.001), were minor and homogeneous with salinities of 5 (6.3 ng rot^{-1}) and 15 (6.0 ng rot^{-1}) psu, but higher and similar at salinities of 25 and 35 psu, with values of 12.4 and 14.2 ng rot^{-1}, respectively.

The results of carbon and nitrogen content as percentage of the dry biomass of rotifers cultivated at different salinities showed a very similar pattern (Table 2). Carbon content was different at different salinities (g.l.=16, F=5.14, P=0.011), but higher and very similar with salinities of 5-25 psu, with average values ranging between 34.6 and 43.6% DB; it was significantly lower (P<0.05) with a salinity of 35 psu (25.8% DB). Similarly, nitrogen content values varied in rotifers grown at the experimental salinities (g.l.=16, F=5.02, P=0.012), but were very homogeneous and higher with salinities of 5, 15 and 25 psu, with average values of 9.6, 8.1 and 8.1% DB, respectively, and with a significantly lower value (P<0.05) at a salinity of 35 psu (5.7% DB). The values of the C:N ratio were between 4.3 and 4.6 and were not statistically different (g.l.=16, F=2.89, P=0.068).

Crude Protein and Energy Content

Figure 3 shows the results of crude protein and energy content of rotifers kept at different salinities. In terms of percentage of the dry biomass, the protein contents of rotifers were statistically different between salinity groups (g.l.=16, F=5.02, P=0.012), with the highest protein content recorded in rotifers

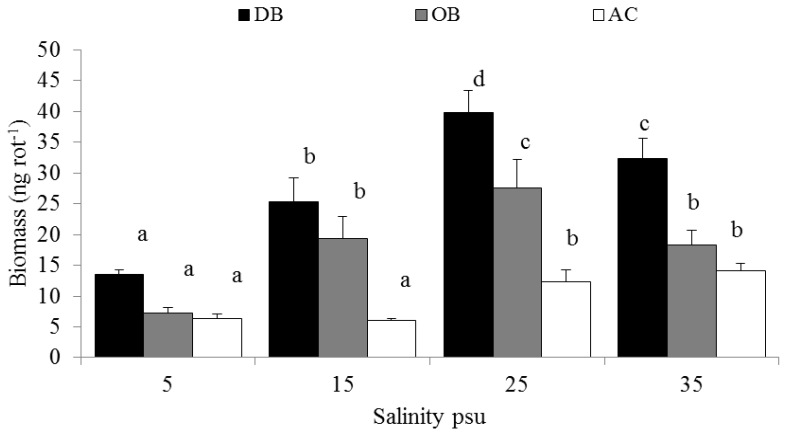

Figure 2. Dry organic biomass, organic biomass (OB) and ash content (AC) for *Proales similis* reared at different salinities (Mean±SD). Different letters indicate significant differences (P<0.05) within salinities.

Table 2. Carbon (C), nitrogen (N) content of dry biomass (DB) (Mean±SD) and C:N ratio for *Proales similis* reared at different salinities (Mean±SD). Different letters indicate significant differences (P<0.05) within columns

Salinity (psu)	C (% BS)	N (% BS)	C:N
5	43.6±9.7[b]	9.6±2.3[b]	4.6±0.3[a]
15	35.9±7.7[b]	8.1±1.5[b]	4.4±0.4[a]
25	34.6±5.8[b]	8.1±1.4[b]	4.3±0.3[a]
35	25.8±4.4[a]	5.7±1.1[a]	4.6±0.2[a]

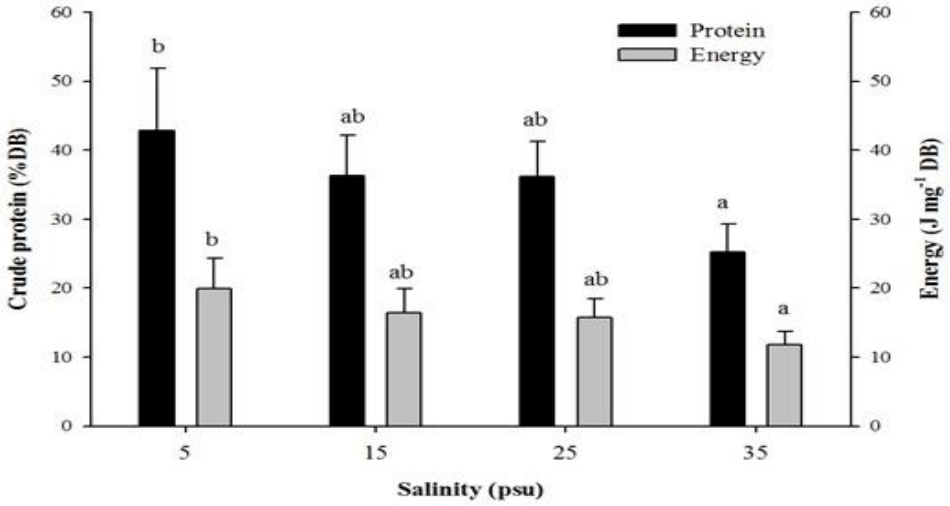

Figure 3. Crude protein and energy content relative to dry biomass (DB) for *P. similis* reared at different salinities. Different letters indicate significant differences (P<0.05) at each salinity per variable.

kept at a salinity of 5 psu (42.8% DB), and the lowest in rotifers kept at a salinity of 35 psu (25.3% DB). The protein contents of rotifers kept at salinities of 15 and 25 psu were intermediate and very similar (36.3 and 36.2% DB, respectively); they were not different (P>0.05) to the values recorded at salinities of 5 and 35 psu.

The changes in the energy content of the rotifers, in response to the various salinities, were similar to those of proteins. The differences were significant (g.l.=16, F=1.15, p=0.011); energy content values were higher for rotifers kept at a salinity of 5 psu (19.9 J mg^{-1} DB) and lower for those kept at a salinity of 35 psu (11.8 J mg^{-1} DB), while at salinities of 15 and 25 psu the energy content was very similar, with values of 16.4 and 15.8 J mg^{-1} DB, respectively, showing no significant differences (P>0.05) with the values recorded at salinities of 5 and 35 psu.

Discussion

In this work, we studied the rotifer *P. similis*, a

highly relevant rotifer because it is a native species of northwestern Mexico that has great potential for use as live feed in the larviculture of marine fish with extremely small mouths, as has been documented by Wullur *et al.,* (2009) and Hagiwara *et al.,* (2014) for *P. similis* from the estuarine waters of Okinawa, Japan. However, strains of the same species with different geographic origins can have different biological characteristics and environmental requirements as a result of different genetic backgrounds (Yin & Zhao, 2008; Malekzadeh-Viayeh *et al.* 2010); thus, it is important to evaluate the potential for growth of the different native strains recently identified. For example, it has been found that salinity affects the lifetime, the reproduction mode and the growth rate of rotifers of the genus *Brachionus* (Bosque, Hernández, Pérez, Todolí, & Oltra, 2001; Hagiwara, Gallardo, Assavaaree, & Koyani., 2001; Kostopoulou, Miliou, Krontira, & Verriopoulus, 2007; Yin & Zhao, 2008). For *P. similis*, Wullur *et al.,* (2009) reported that in the salinity range of 2-30 psu and in serial cultures, the highest densities were recorded at salinities of 2 and 15 psu (500 and 360 rot mL^{-1}, respectively), while the lowest densities were recorded at salinities of 20-30 psu (<120 rot mL^{-1}), suggesting that salinities of 2-15 psu are the best for the growth of *P. similis* (which coincides with the salinities of its original natural environment) under laboratory conditions, and that the lower growth recorded at salinities >25 psu was due to the increase in energy demand for osmoregulation and a decrease in the filtration rate. However, in mass cultures of rotifers acclimated to 25 °C and 25 psu, there were maximum densities of up to 2400 rot mL^{-1} and an average r of 0.42 day^{-1}.

Coinciding with the study of Wullur *et al.,* (2009), the results of this investigation indicated that salinities in the range of 5-35 psu significantly influenced the maximum density (Dmax) after 8 days of culture. The lowest value was recorded with a salinity of 35 psu (1703 rot mL^{-1}, r=0.46 day^{-1}) and the highest values with salinities below 35 psu (2488 to 2560 rot mL^{-1}, r=0.50 to 0.51 $days^{-1}$). These data are very similar to those recorded by Wullur *et al.,* (2009) in mass cultures at 25 psu; however, in the present study, the salinity range tested of 5-35 psu did not significantly affect the intrinsic growth rate and the duplication time of *P. similis*. In fact, the smallest Dmax recorded at a salinity of 35 psu was between 31 and 33% lower than at the other salinities; thus, it is probable that *P. similis* responds favorably to a salinity of 5 to 35 psu due to its euryhaline nature. We therefore consider it appropriate to establish that *P. similis* is able to grow in a wide range of salinity, confirming that the species is euryhaline. This is corroborated by the works of De Smet (1996), Sørensen (2001), Sørensen and Giribet (2006) and Segers (2007), which indicate that *P. similis* is a cosmopolitan species that has been reported in marine, estuarine and brackish waters, while Maitland (1977) reported it in British freshwater lakes, and Wallace, Walsh, Schröder, Rico-Martínez, and Rios-Arana (2008) reported it for the first time in saline waters of the Chihuahuan Desert, Mexico. Brain and Koste (1993) point out that the species tolerates salinities of up to 98 psu. Only one work fails to agree, as Fontaneto, De Smet, and Ricci (2006) classifies *P. similis* as strictly saline but not euryhaline.

In this study, it was possible to determine that the culture salinities evaluated differed with respect to the effect on the protein and energy content of this rotifer. The protein content of *P. similis* fluctuated between 25.3 and 42.8% DB, while the energy content was between 11.8 and 19.9 J mg^{-1} DB. In both cases, the differences were significant only between the salinities of 5 and 35 psu, with intermediate values for salinities of 15 and 25 psu. Considering that other studies reported that the protein contents of rotifers of the genus *Brachionus* used as live feed in aquaculture are in the range of 28 to 63% DB (Øie & Olsen, 1997), while the energy contents have been reported in the range of 15-24 J mg^{-1} DB (Yúfera & Pascual, 1989; Yúfera, Parra, & Pascual, 1997), we established that under the conditions tested, the values recorded in this study were within the range reported in the literature for other species of rotifers used as live feed, except for the lower values of protein and energy content recorded at the salinity of 35 psu. If the similarities with rotifers of the genus *Brachionus* are maintained, the reduction in protein and energy content at a salinity of 35 psu could indicate that at that salinity *P. similis* has a higher energy and protein expenditure due to the increased costs of osmoregulation (Lowe *et al.,* 2005), as was also reported by Wullur *et al.* (2009) for *P. similis*; this seems to be reflected in one of the population parameters evaluated, since a lower Dmax was observed at this salinity, without affecting the intrinsic growth rate and the population duplication time. Our estimates of the crude protein and energy content of *P. similis* were comparable to those reported for other species of rotifers used as live feed in aquaculture, suggesting that carbon and nitrogen content, and their respective energy and crude protein equivalents, provide reliable estimates. Judging by the results of this study, it is possible to grow the rotifer *P. similis* in high densities in the range of salinities tested, with an optimum result at salinities lower than 35 psu, even at 2 psu, as reported by Wullur *et al.* (2009), and at 35 psu, as long as the rotifers are acclimated for a reasonable time. This is relevant, because it highlights its viability as first live feed for marine fish larvae and for larvae requiring salinities close to freshwater. In addition, the protein and energy content, at least at salinities of 5-25 psu, was very stable, indicating that *P. similis* is biochemically and energetically similar to other species used as live feed and can be successfully exploited for purposes of live feed for marine larval rearing; moreover, in the

future, given its size (total length of 90±1.94 µm, 56±1.66 µm wide), it will probably contribute to the success of rearing marine fish larvae with extremely small mouths, which are not able to feed on *B. rotundiformis.*

The intrinsic growth rate (r) is a variable that integrates survival, fecundity, development time and reproduction (Malekzadeh-Viayeh *et al*., 2010), while duplication time (Dt) allows to predict the abundance of rotifers at any point of the growth curve and is similar to the generation time (average time between the birth of parents and the birth of their descendants) (Gillooly, 2000). The maximum population density indicates the abundance that can be supported by the culture conditions as a reflection of the changes in survival and reproduction; it also outlines the acclimation history of the population to the experimental conditions (Krebs, 1985). Since r and Dt were not affected by a salinity of 35 psu, and r includes survival and reproduction, it is likely that the acclimation time of one week was insufficient for the rotifers to show a better performance. However, the C:N ratio does not seem to reflect this pattern, since no proportional change was observed in the carbon and protein content of *P. similis* when salinity changed; the C:N ratio remained nearly constant, with values between 4.3 and 4.6, although it showed significantly lower carbon and nitrogen values at a salinity of 35 psu.

Finally, it is interesting to note that despite the homogeneity of the carbon and nitrogen content, and of their calorific and crude protein equivalents, at salinities of 5 to 25 psu the dry biomass and organic biomass were significantly increased, while the ash content per rotifer (ng rot^{-1}) was lower and remained without apparent changes at salinities of 5-15 psu, and then increased, with very similar values, at salinities of 25 and 35 psu; representing 46.85, 23..8%, 31.0% and 43.7% of total dry biomass at 5, 15, 25 and 35 psu, respectively. The above suggests that the observed variability in protein and calories content in the salinity range tested can be attributed, at least in part, to changes in ash content in response to changes of salinity, as was also reported for rotifers of the genus *Brachionus* by Yúfera and Pascual (1989). Ash content is a measure of the amount of inorganic minerals present in the sample and is generally between 3.0 and 7.8% of the dry biomass in different rotifer species (Watanabe, Kitajima, & Fujita, 1983; Jeeja, Joseph, & Malej, 2011); however, in our samples of *P. similis*, the ash contents were between 23.8 and 46.85% DB, which contrast with the values reported for other species of rotifers, but are very similar to those reported for gelatinous species (>50% DB) (Clarke, Holms, & Gore, 1992; Kiørboe, 2013), probably because of the close relationship of the family Proalidae with other gelatinous organism or jelly plankton as stated by Dumont (2007). Hirst and Lucas (1998), and Kogovsek, Tinta, Klum, and Malej (2014), mentioned that it is common that gelatinous species show an increase in dry biomass and ash content with increasing salinity. This may have important nutritional implications if *P. similis* is used for larval feeding, since it has been reported that gelatinous species have low nutritional quality (Doyle, Houghton, McDevitt, Davenport, & Hays, 2007); however, this aspect requires further investigation in the different strains of *P. similis* from different geographical regions.

In conclusion, the results of this study showed that the strain of the rotifer *P. similis* native of northwestern Mexico presents growth rates, population duplication times and maximum densities similar to those reported for another strain from Japanese estuaries. Furthermore, the protein and energy contents were comparable to those obtained for the genus *Brachionus*, which is widely used in aquaculture to feed fish and crustacean larvae. Also, the rotifer *P. similis* is viable as the first live feed for marine fish larvae and for larvae that require salinities close to freshwater, since they can tolerate a wide range of salinity. The protein and energy content, at least at salinities of 5-25 psu, was very stable, indicating that *P. similis* is biochemically and energetically similar to other species used as live feed and can be successfully exploited for purposes of live feed for marine larval rearing; moreover, in the future, given its size (total length of 90±1.94 µm, 56.±1.66 µm wide), it will probably contribute to the success of rearing marine fish larvae with extremely small mouths, which are not able to feed on *B. rotundiformis*. However, the high ash content recorded in the issue of *P. similis* requires further investigation, since it reflects some resemblance to gelatinous species, and it has been reported that gelatinous species have low nutritional quality, which could have important nutritional implications if *P. similis* is used in larval feeding.

Acknowledgements

Financial support for this work was provided by the Mexican Research Network Program PRODEP-SEP 103.5/15/11050 and the Programa de Fomento y Apoyos a Proyectos de Investigación (PROFAPI 2013/171) of the Universidad Autónoma de Sinaloa, we are indebted to Rene Bojórquez for his technical assistance in the molecular analysis for species identification.

References

Bosque, T., Hernández, R., Pérez R., Todolí, R., & Oltra, R. (2001). Effects of salinity, temperature and food level on the demographic characteristics of the seawater rotifer *Synchaeta littoralis* Rousselet. *Journal of Experimental Marine Biology and Ecology*, 258(1), 55-64. http://dx.doi.org/10.1016/S0022-0981(00)00345-2

Brain, C.K., & Koste, W. (1993). Rotifers of the genus *Proales* from saline springs in the Namib desert, with

the description of a new species. *Hydrobiologia*, 255(1), 449-454. http://dx.doi.org/10.1007/BF00025872

Braithwaite, V.A., & Salvanes, A.G.V., (2010). Aquaculture and restocking: implications for conservation and welfare. *Animal Welfare*, 19(2), 139-149.

Chigbu, P., & Suchar, V.A. (2006). Isolation and culture of the marine rotifer, *Colurella dicentra* (Gosse, 1887), from a Mississippi Gulf Coast estuary. *Aquaculture Research*, 37(14), 1400-1405. http://dx.doi.org/10.1111/j.1365-2109.2006.01572.x

Ciros-Pérez, J., Gómez, A., & Serra, M. (2001). On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. *Journal of Plankton Research*, 23(12), 1311-1328. http://dx.doi.org/10.1093/plankt/23.12.1311

Clarke, A., Holms, L.J., & Gore, D.J. (1992). Proximate and elemental composition of gelatinous zooplankton from the Southern Ocean. *Journal of Experimental Marine Biology and Ecology*, 155(1), 55-68. http://dx.doi.org/10.1016/0022-0981(92)90027-8

Das, P., Mandal, S.C., Bhagabati, S.K., Akhtar, M.S., & Singh, S.K. (2012). Important live food organisms and their role in aquaculture. In: J.K. Sundaray, S. Munilkumar, R.K. Mohanty, & Otta, S.K. (Eds.), *Frontiers in Aquaculture*. New Delhi, India, Narendra Publishing House, pp 69-86.

De Smet, W.H. (1996). Rotifera 4: The Proalidae (Monogononta). In: H.J. Dumont, & T. Nogrady (Eds.), *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 12*. The Hague, The Netherlands: SPB Academic Publishing pp. 1-102.

Doyle, T.K., Houghton, J.D.R., McDevitt, R., Davenport, J., & Hays, G.C. (2007). The energy density of jellyfish: Estimates from bomb-calorimetry and proximate-composition. *Journal of Experimental Marine Biology and Ecology*, 343(2), 239-252. http://dx.doi.org/10.1016/j.jembe.2006.12.010

Dumont, H.J. (2007). Rotifers, the jelly plankton of freshwater. *Hydrobiologia*, 593(1), 59-66. http://dx.doi.org/10.1007/s10750-007-9047-8

Fontaneto, D., De Smet, W.H., & Ricci, C. (2006). Rotifers in saltwater environments, reevaluation of an inconspicuous taxon. *Journal of the Marine Biological Association of the United Kingdom*, 86(04), 623-656. http://dx.doi.org/10.1017/S0025315406013531

Gillooly, J.M. (2000). Effect of body size and temperature on generation time in zooplankton. *Journal of Plankton Research*, 22(2), 241-251. http://dx.doi.org/10.1093/plankt/22.2.241

Guillard, R.R.L., & Ryther, J.H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Canadian Journal of Microbiology*, 8(2), 229-239. http://dx.doi.org/10.1139/m62-029

Hagiwara, A., Wullur, S., Marcial, H.S., Hirai, N., & Sakakura, Y. (2014). Euryhaline rotifer *Proales similis* as initial live food for rearing fish with small mouth. *Aquaculture*, 432, 470-474. http://dx.doi.org/10.1016/j.aquaculture.2014.03.034

Hagiwara, A., Gallardo, W.G., Assavaaree, M., Kotani, T., & de Araujo, A.B. (2001). Live food production in Japan: recent progress and future aspects. *Aquaculture*, 200(1), 111-127. http://dx.doi.org/10.1016/S0044-8486(01)00696-2

Hirai, N., Koiso, M., Teruya, K., Kobayashi, M., Takebe, T., Sato, T., Nakamura, K., Goto, T., & Hagiwara, A. (2012). Rearing conditions for Humphead Wrasse *Cheilinus undulatus* larvae, and introduction of the minute rotifer *Proales similis* as an initial live food. *Journal of Fisheries Technology*, 4(2), 57-64.

Hirst, A.G., & Lucas, C.H. (1998). Salinity influences body weight quantification in the scyphomedusa *Aurelia aurita*: Important implications for body weight determination in gelatinous zooplankton. *Marine Ecology Progress Series*, 165, 259-269. http://dx.doi.org/10.3354/meps165259

Jeeja, P.K., Joseph, I., & Raj, R.P. (2011). Nutritional composition of rotifer (*Brachionus plicatilis* Muller) cultured using selected natural diets. *Indian Journal of Fisheries*, 58, 59-65.

Kiørboe, T. (2013) Zooplankton body composition. *Limnology and Oceanography*, 58(5), 1843-1850. http://dx.doi.org/10.4319/lo.2013.58.5.1843

Kogovšek, T., Tinta, T., Klun, K., & Malej, A. (2014). Jellyfish biochemical composition: importance of standardized sample processing. *Marine Ecology Progress Series*, 510, 275-288. http://dx.doi.org/10.3354/meps10959

Koste, W. (1978). *Rotatoria*. Die Rädertiere Mitteleuropas. Borntraeger, Berlin.673 pp.

Kostopoulou, V., Miliou, H., Krontira, Y., & Verriopoulos, G. (2007). Mixis in rotifers of the lineage 'Nevada' belonging to the *Brachionus plicatilis* species complex, under different feeding regimes. *Aquaculture Research*, 38(10), 1093-1105. http://dx.doi.org/10.1111/j.1365-2109.2007.01783.x.

Krebs, C.J. (1985). Ecology; the experimental analysis of distribution and abundance. New York, USA, .Harper and Row, Publishers, 800 pp.

Lahope, H.B., Wullur, S., Rimper, J., Pangkey, H., & Rumengan, I.F.M. (2013). Minute rotifers from estuarine waters in North Sulawesi, Indonesia, and their potentials as starting food for fish larvae. *Jurnal Perikanan dan Kelautan Tropics*, 9, 8-12.

Lowe, C.D., Kemp, S.J., Bates, A.D., & Montagnes, D.J.S. (2005). Evidence that the rotifer *Brachionus plicatilis* is not an osmoconformer. *Marine Biology*, 146(5), 923-929. http://dx.doi.org/10.1007/s00227-004-1501-9

Maitland, P.S. (1977). A coded checklist of animals occurring in fresh water in the British Isles, Edinburgh, UK,: Institute of Terrestrial Ecology, 76 p.

Malekzadeh-Viayeh, R., Mohammadi, H., & Shafiei, A.B. (2010). Population Growth of Six Iranian *Brachionus* Rotifer Strains in Response to Salinity and Food Type. *International Review of Hydrobiology*, 95(6), 461–470. http://dx.doi.org/10.1002/iroh.201011263.

Malekzadeh-Viayeh, R. (2012). Interactive effects of food and salinity on the reproductive and growth indices of two *Brachionus* rotifer strains from Iran. *Iranian Journal of Science and Technology*, 36(A3), 245.

Miracle, M.R., & Serra, M. (1989). Salinity and temperature influence in rotifer life history characteristics. *Hydrobiologia*, 186(1), 81-102. http://dx.doi.org/10.1007/BF00048900.

Nagano, N., Iwatsuki, Y., Kamiyama, T., Shimzu, H., & Nakata, H. (2000). Ciliated protozoans as food for first-feeding larval grouper, *Epinephelus septemfasciatus*: Laboratory experiment. *Plankton Biology and Ecology*, 47(2), 93-99.

Nakagawa, Y., Senoo, S., & Miyashita, S. (2007). Protozoa and diatoms as primary food sources for larvae of the blue spotted snapper *Lutjanus rivulatus*. *Aquaculture Science*, 55(1), 25-130. http://dx.doi.org/10.11233/aquaculturesci1953.55.125

Øie, G., Reitan, K.I., & Olsen, Y. (1994). Comparison of rotifer culture quality with yeast plus oil and algal-based cultivation diets. *Aquaculture International*, 2(4), 225-238. http://dx.doi.org/10.1007/BF00123431

Øie, G., & Olsen, Y. (1997). Protein and lipid content of the rotifer *Brachionus plicatilis* during variable growth and feeding condition. In *Live Food in Aquaculture* (pp. 251-258). Springer Netherlands.

Olivotto,, I., Holt, S.A., Carnevali, O., & Holt, G.J. (2006). Spawning, early development, and first feeding in the lemonpeel angelfish *Centropyge flavissimus*. *Aquaculture*, 253(1), 270-278. http://dx.doi.org/10.1016/j.aquaculture.2004.12.009

Rimmer, M. (2000). Review of grouper hatchery technology. *SPC Live Reef Fish Information Bulletin*, 7, 14-19.

Rodríguez-Montes de Oca, G.A., Medina-Hernández, E.A., Velázquez-Sandoval, J., López-López, V.V., Román-Reyes, J.C., Dabrowski, K., & Haws, M.C. (2012). Production of "Chame"(*Dormitator latifrons*, Pisces: Eleotridae) larvae using GnRHa and LHRHa. *Revista Colombiana de Ciencias Pecuarias*, 25(3), 422-429.

Román-Reyes J.C., Castañeda-Rodríguez D.O., Castillo-Urreta H., Bojórquez-Dominguez R., & Rodríguez-Montes de Oca G.A. 2014. Dinámica poblacional del rotífero *Brachionus ibericus* aislado de estanques para camarón, alimentado con diferentes dietas. *Latin American Journal of Aquatic Research*, 42(5), 1159-1168. http://dx.doi.org/ 10.3856/vol42-issue5-fulltext-19

Salonen, K., Sarvala, J., Hakala, I., & Viljanen M.L. (1976). The relation of energy and organic carbon in aquatic invertebrates. *Limnology and Oceanography*, 21(5), 724-730. http://dx.doi.org/10.4319/lo.1976.21.5.0724

Schipp, G.P., Bosmans, J.M.P., & Marshall, A.J. (1999). A method for hatchery culture of tropical calanoid copepoda, *Acartia* spp. *Aquaculture*, 174(1), 81-88. http://dx.doi.org/10.1016/S0044-8486(98)00508-0

Segers, H. (1995). The Lecanidae (Monogononta). In H.J. Dumont, T. Nogrady (Eds.) *Rotifera 2. Guides to the identification of the microinvertebrates of the continental waters of the world, 6*. The Hague, Netherlands SPB Academic Publishing, p 1-226.

Segers, H. (2007). Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. *Zootaxa*, 1564, 1-104.

Sørensen, M.V. (2001). The rotifer fauna of Bermuda, including notes on the associated meiofauna and the description of a new species of *Encentrum* (Rotifera: Monogononta: Ploima). *Proceedings of The Biological Society of Washington*, 114, 725-736.

Sørensen, M.V., & Giribet, G. 2006. A modern approach to rotiferan phylogeny: Combining morphological and molecular data. Molecular *Phylogenetics and Evolution*, 40(2), 585-608. http://dx.doi.org/10.1016/j.ympev.2006.04.001

Srivastava, A., Hamre, K., Stoss, J., Chakrabarti, R., & Tonheim, S.K. (2006). Protein content and amino acid composition of the live feed rotifer (*Brachionus plicatilis*): With emphasis on the water soluble fraction. *Aquaculture*, 254(1), 534-543. http://dx.doi.org/10.1016/j.aquaculture.2005.11.014

Takeuchi, T. (2014). Progress on larval and juvenile nutrition to improve the quality and health of seawater fish: a review. *Fisheries Science*, 80(3), 389-403. http://dx.doi.org/10.1007/s12562-014-0744-8

Toledo, J.D., Golez, M.S., Doi, M., & Ohno, A. (1999). Use of copepod nauplii during early feeding stage of grouper, *Epinephelus coiodies*. *Fisheries Science*, 65(3), 390-397. http://dx.doi.org/10.2331/fishsci.65.390

Tomoda, T., Furuita, H., Kamoshida, M., Kurogi, H., Shibuno, T., Tanaka, H., & Tezuka, N. (2014). Nutritional enrichment trials of the minute rotifer *Proales similis* using commercial materials. *Journal of Fisheries Technology*, 6(2), 179-184.

Treece,, G., & Davis; D.A. (2000). Culture of Small Zooplankters for the Feeding of Larval Fish, University of Florida, Florida, Southern Regional Aquaculture Center Publication No. 701, USA. 8 pp.

Wallace, R.L., Walsh, E.J., Schröder, T., Rico-Martínez, R., & Rios-Arana, J.V. (2008). Species composition and distribution of rotifers in Chihuahuan Desert waters of México: is everything everywhere?. *Verhandlungen des Internationalen Verein Limnologie*, 30(1), 73-76.

Wang, Y., Li, L., Cui, G., & Lu, W. (2013). Ontogenesis from embryo to juvenile and salinity tolerance of Japanese devil stinger *Inimicus japonicus* during early life stage. *SpringerPlus*, 2(1), 2-13. http://dx.doi.org/10.1186/2193-1801-2-289

Watanabe, T., Kitajima, C., & Fujita S. (1983). Nutritional values of live organisms used in Japan for mass propagation of fish: a review. *Aquaculture*, 34(1), 115-143. http://dx.doi.org/10.1016/0044-8486(83)90296-X

Wullur, S., Sakakura, Y., & Hagiwara, A. (2009). The minute monogonont rotifer *Proales similis* de Beauchamp: Culture and feeding to small mouth marine fish larvae. *Aquaculture*, 293(1), 62-67. http://dx.doi.org/10.1016/j.aquaculture.2009.04.011

Wullur, S., Sakakura, Y., & Hagiwara, A. (2011). Application of the minute monogonont rotifer *Proales similis* de Beauchamp in larval rearing of seven-band grouper *Epinephelus septemfasciatus*. *Aquaculture* 315(3), 355-360. http://dx.doi.org/10.1016/j.aquaculture.2011.02.025

Wullur, S., Yoshimatsu, T., Tanaka, H., Ohtani, M., Sakakura, Y., Kim, H.J., & Hagiwara, A. (2013). Ingestion by Japanese eel *Anguilla japonica* larvae on various minute zooplanktons. *Aquaculture Science*, 61(4), 341-347.

Yin, X.W., and Zhao, W. (2008). Studies on life history characteristics of *Brachionus plicatilis* O. F. Müller (Rotifera) in relation to temperature, salinity and food algae. *Aquatic Ecology*, 42(1), 165-176. http://dx.doi.org/10.1007/s10452-007-9092-4

Yúfera, M., & Pascual, E. (1989). Biomass and elemental composition (CHN) of the rotifer *Brachionus plicatilis* cultured as larval food. *Hydrobiologia*, *186*(1), 371-374. . http://dx.doi.org/10.1007/10.1007/BF00048934

Yúfera, M., Parra, G., & Pascual, E. (1997). Energy content of rotifers (*Brachionus plicatilis* and *Brachionus rotundiformis*) in relation to temperature. In *Live Food in Aquaculture* (pp. 83-87). Springer Netherlands.

Zar J.H. (1999). Biostatistical Analysis.4th ed. Prentice Hall, Inc. 660 p.