COMPARATIVE QUALITY OF ROTIFERS AND COPEPODS AS FOODS FOR LARVAL FISHES

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ABSTRACT

The rotifer *Brachionus plicatilis* and the copepod *Tigriopus californicus* are easily cultured and commonly used as foods for larval fishes. Sizes of rotifers, nauplii, and copepodites, ranging in width from 74 to 221 μm, were related to their weight, volume, and caloric content. Between the smallest and largest size classes—a width increase of two times—rotifer dry weight increased from 0.10 to 0.47 μg/animal, and naupliar dry weight increased from 0.04 to 0.38 μg/animal. An individual rotifer contained more calories than a nauplius of the same width even though the organic tissue fraction of the rotifers had a lower caloric value (4.8 cal/mg) than that of the copepods (5.9 cal/mg). *Tigriopus* nauplii weighed less per unit width and contained more ash than rotifers. These width-specific differences in caloric content should be considered in any bioenergetic study in which rotifers and copepod nauplii are used as foods.

RESUMEN

El rotífero *Brachionus plicatilis* y el copépodo *Tigriopus californicus* son de fácil cultivo y son frecuentemente utilizados como alimento para larvas de peces. Las dimensiones transversales de los rotíferos, nauplios y copepoditos oscilan entre 74 y 225 μm. Estas tallas fueron relacionadas con el peso, volumen y valor calorico respectivo en cada grupo. Entre las tallas más pequeñas y las mayores, si la anchura se duplica, el peso seco de los rotíferos aumenta de 0.10 a 0.47 μg, y el de los nauplios de 0.04 a 0.38 μg por ejemplar. Un rotífero contiene más calorías que un nauplio del mismo ancho, aunque la fraccíon de tejido orgánico del rotífero tiene un valor calórico inferior (4.8 cal/mg) que en los copépodos (5.9 cal/mg). Los nauplios de *Tigriopus* pesaron menos por unidad de anchura y produjeron más cenizas que los rotíferos. Estas diferencias en valor calórico específicas en relación a la dimensión transversal de los organismos, debiera ser consideradas en las estimaciones bionergéticas, cuando rotíferos y nauplios son utilizados como alimento.

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INTRODUCTION

In studies of larval fish energetics, it is necessary to know dry weights and caloric values of prey items. We have determined these values for the rotifer *Brachionus plicatilis* and for the nauplii and copepodites of *Tigriopus californicus*, a harpacticoid copepod, as part of a study on effect of diet on growth of larval northern anchovy, *Engraulis mordax*. Rotifers are widely used as food for larval fish in laboratories (Theilacker and McMaster 1971; Solangi and Ogle 1977; Fontaine and Revera 1980; Hunter 1981); harpacticoid copepods are an important food for many larval fish in the field (Mason 1974; Arthur 1976; Schmidt-Moser and Westphal 1980) and are also used as prey in laboratories (Kinne 1977; Kahan et al. 1982; Hunter MS[1]). We cultured *Brachionus* using the methods of Theilacker and McMaster (1971), and *Tigriopus* using the technique followed at the Southwest Fisheries Center, La Jolla, California.

Copepod biomass has been determined with several methods and expressed in different units (Table 1). Copepod total length and prosome length have been used as predictors for both dry and wet weight of Formalin-preserved adults (Pertsova 1967; Grusov and Alekseyeva 1970; Durbin and Durbin 1978; Feigenbaum 1979), Formalin-preserved copepodites (Mullin and Brooks 1967; Durbin and Durbin 1978), and Formalin-preserved nauplii (Durbin and Durbin 1978). Yet width is the dimension that limits fish larvae's selection of prey (Beyer 1980; Hunter 1981), and width is also a more accurate predictor of copepod wet weight than is length (Pearre 1980). Because copepods lose weight when preserved in Formalin (Ahlstrom and Thrailkill 1963; Omori 1970, 1978), and the dry weight lost varies among species (Omori 1970, 1978; Durbin and Durbin 1978), a width-weight relation for fresh samples is needed. Fresh dry weights available for copepods have been related to length (Comita et al. 1966) and to stage (Paffenhöfer 1971), or they have been given as a mean weight for a composite sample of field-collected nauplii and copepodites (Houde and Schekter 1981). The fresh dry weights reported for

[1]Hunter, J.R. Synopsis of culture methods for marine fish larvae. Presented at the Ahlstrom Memorial Symposium, La Jolla, California, August 1983. Manuscript.

TABLE 1
Copepod Weight and Caloric Data

	Life stage[1]: 1-naup; 2-cop; 3-cop-V; 4-adults; 5-mixed	Fresh sample	Predictor of weight (weight = 1-dry; 2-wet; 3-carbon weight) Length	Stage	Caloric content	Ash content	Reference
Individual species							
Acartia clausi	1;2;4	–	1	1	–	–	Durbin & Durbin 1978
Acartia tonsa	1;2;4	–	1	1	–	–	Heinle 1966
Calanus cristatus	3;4	–	1;2	1;2[2]	–	+	Omori 1970
Calanus finmarchicus	3;4	+	–	1	+	+	Comita & Schindler 1963
Calanus finmarchicus	3;4	+	1	1	+	–	Comita et al. 1966
Calanus helgolandicus	5	+	–	–	+	–[3]	Slobodkin & Richman 1961
Calanus helgolandicus	1;2;4	+	–	3	–	–	Mullin & Brooks 1970a; 1970b
Calanus helgolandicus	1;2;4	+	–	3	–	–	Paffenhöfer 1971
Calanus pacificus	1	+	–	3	–	–	Fernandez 1979
Centropages typicus	2	–	–	1;3	+	+	Razouls 1977
Rhincalanus nastus	2	–	1;3	–	–	–	Mullin & Brooks 1967
Rhincalanus nastus	2	+	–	3	–	–	Mullin & Brooks 1970a
Temora stylifera	2	–	–	1;3	+	+	Razouls 1977
Tigriopus californicus	5	+	–	–	+	–[3]	Slobodkin & Richman 1961
Individual genus							
Pseudocalanus	1;2;4	–	1	1;3	+	+	Corkett & McLaren 1978[4]
Many species treated individually							
	4	–	1	–	–	–	Conover 1959
	3;4	+	–	1	–	–	Conover 1960
	2;4	–	2	2	–	–	Pertsova 1967
	2;4	–	2	2	–	–	Krylov 1968
	3;4	–	2	2	–	–	Grusov & Alekseyeva 1970
	1;2;4	?	–	1	–	–	Hargrave & Geen 1970
	4	–	–	–	+	+	Laurence 1976
	3;4	+	1;3	–	–	+	Omori 1978
	1;2	+	1	–	–	–[3]	Breteler et al. 1982
Unidentified copepods or mixed samples							
	5[2]	+	–	–	–	–	Beers 1966
	5	–	1	–	–	–	Feigenbaum 1979
	5[5]	+	–	–	–	–	Houde & Schekter 1981
	5[6]	–	–	–	–	–	Hanson et al. 1982

[1]Cop = copepodite; cop-V = stage V copepodite
[2]Copepod carbon content is given as a percentage of dry weight
[3]Ash content is corrected for, but not given
[4]Review paper
[5]Copepod dry weight is related to mesh size used for sample (see text)
[6]Mean dry weight is given

rotifers (Theilacker and McMaster 1971; Doohan 1973; Eldridge et al. 1977) were given as averages for laboratory populations, and they do not reflect size-specific weight.

Not only is dry weight lower after Formalin preservation, but significant amounts of carbon and nitrogen are also lost from copepod tissues (Durbin and Durbin 1978; Omori 1978). Thus, calorimetric measurements are most accurately performed on fresh samples. Calorimetric values are available for fresh samples of adult calanoid copepods (Slobodkin and Richman 1961; Comita and Schindler 1963; Comita et al. 1966) and adult harpacticoids (Slobodkin and Richman 1961). No information was available on the width-specific dry weight and caloric content of fresh samples of rotifers, copepod nauplii, and copepodites that are commonly used as foods for larval fishes.

TABLE 2
Prey Characteristics

Prey	Width class µm	Per individual: Dry[1] weight µg (SE)	Volume $\times 10^6 \mu m^3$	Caloric value $\times 10^{-3}$ cal
Brachionus plicatilis (4.4 cal/mg)[2]				
Rotifers	74-110	0.10 (.01)	0.65	0.44
	110-147	0.22 (.04)	1.73	0.97
	147-184	0.41 (.06)	2.96	1.80
	184-195	0.47 (.08)	3.99	2.07
Tigriopus californicus (4.9 cal/mg)[2]				
Nauplii	74-110	0.04 (.01)	0.20	0.20
	110-147	0.13 (.01)	0.55	0.64
	147-184	0.25 (.01)	1.17	1.23
	184-195	0.38 (.00)[3]	1.77	1.86
Copepodites				
	147-184	0.63 (.15)	3.38	3.09
	184-221	1.20 (.26)	6.21	5.88

[1]Regression coefficients are used for average dry weights.
[2]Caloric value for total tissue dry weight; see text.
[3]<0.01.

METHODS

Preparation of Rotifers

We harvested rotifers from cultures and placed them overnight in a one-liter cylinder containing seawater and algae. This procedure allowed debris to settle and insured that the rotifers were in a good nutritional state when sampled. Thus the nutritional characteristics of the rotifers when dried and weighed should be comparable to their state when used as food. We siphoned debris-free rotifers through a series of nitex screens (ranging in size from 44 µm to 194 µm), retaining samples in which the major component was animals of a single width class (Table 2). Pure samples (n = 3) were obtained for the 74-110 µm rotifer width class, but the other sieved samples (n = 25) were slightly contaminated by rotifers from adjacent size classes. For these samples, an aliquot was counted to estimate total numbers (the coefficient of variation for the aliquots was between .17 and .20) and numbers belonging to each width class. Next, we poured a volume containing 2,500-5,000 animals (usually about 30 ml) onto a preweighed Nucleopore filter (Durbin and Durbin 1978). To eliminate salt crystals, we rinsed the samples with 30 ml of isotonic ammonium formate (3.4%), which sublimates when heated, leaving no residue. Blanks of seawater and ammonium formate were run through filters and used to correct the final weights.

We estimated width-specific dry weight of the rotifers belonging to the three slightly contaminated width classes by using a multiple regression ($y = a_1x_1 + a_2x_2 + a_3x_3$) in which y was the total dry weight of the sample, x_1, x_2, and x_3 were the relative frequencies of the width classes, and a_1, a_2, and a_3 were the width-specific average dry weights (Draper and Smith 1966). We used the midpoint of the width range for the four width classes and corrected the total weight for the frequency of eggs, assuming egg weight was the same as the weight of the smallest rotifer, 0.10 µg, which agrees with an unpreserved egg weight of 0.092 µg determined for the same species by Doohan (1973).

Preparation of Copepods

Copepods were difficult to sieve because the appendages of the live animals caught on the mesh of the nets. Thus carapace width was not the limiting factor in sieving. We found that anesthetizing the animals with dilute MS222 (0.06% MS222 in seawater) relaxed the appendages and permitted more effective sieving. The anesthetized animals were quickly revived in sea water. We rinsed sieved animals in ammonium formate solution and hand sorted them to width classes (Table 2) on a slide, forming nauplii and copepodites into piles of 200 to 400 animals. We sieved and sorted 10 copepod samples. Five of the sorted samples were composed of a single size (width) class, and the remaining samples were of two size groups. To estimate size-specific weights for copepods, we followed the same multiple regression procedure used for rotifers.

We placed slides of copepods and Nucleopore filters with rotifers in a 60°C oven and dried them overnight to constant weight (Lovegrove 1966). We then removed the piles of copepods from the slides by using a single-edged razor blade and weighed the copepod samples, as well as the rotifer samples with filters, on a Cahn electrobalance to ± 2 µg.

Calculation of Rotifer and Copepod Volumes

Rotifers and nauplii have similar length-width ratios, but they differ in body form and thus in volume. We approximated rotifer volumes as ellipsoids, naupliar volumes as oblate spheroids (dorsoventrally compressed spheres), and copepodite volumes as the sum of a dorsoventrally compressed and elongated ellipsoid (for the prosome or cephalothorax) plus a cylinder (for the urosome or abdomen). After measuring each dimension of individual animals (n = 30 for each width class) to find the relative ratios of the dimensions, we calculated volumes using the midpoint of the width range (Table 2).

We used a Phillipson oxygen microbomb calorimeter to measure caloric content; samples weighed from 2 to 4 mg. Ash was estimated by incinerating dried specimens at 525°C overnight. We hand sorted about 10,000 nauplii and 1,000 copepodites for each calorimetric and ash determination.

RESULTS AND DISCUSSION

Rotifer Dry Weight and Caloric Value

Rotifer dry weight estimates range between 0.10 and 0.47 μg, depending on body width (Table 2). To compare these estimates with the fresh dry weights of *B. plicatilis* given as averages for laboratory populations (Theilacker and McMaster 1971; Doohan 1973; Eldridge et al. 1977), we calculated an average weight using the width-specific weights determined in this study and Theilacker and McMaster's (1971) width-frequency information. The calculated average weight, 0.20 μg, was similar to the average weight of 0.16 μg reported by Theilacker and McMaster (1971). Doohan (1973) also obtained a mean individual dry weight of 0.158 μg. The estimate given by Eldridge et al. (1977) was 0.905 μg, twice the weight of rotifers in our largest width class (Table 2). They observed a large number of egg-bearing females in their samples and did not adjust for egg weight, which may partially explain this weight difference.

We used ash content of 7.8 ± 2.0% dry weight in our expressions of ash-free caloric content (Table 3). Watanabe et al. (1983) give data for *B. plicatilis* from which we calculated a somewhat lower ash content (5.32 ± 1.83% dry weight, n = 2). Theilacker and McMaster's (1971) estimated caloric value for *Brachionus* was 5.3 ± 0.1 cal/mg ash-free dry weight, and we found a caloric value of 4.8 ± 0.3 cal/mg ash-free dry weight (Table 3). This variation in ash-free caloric values may simply reflect differences in ash content. Apparently, weight and caloric content among *Brachionus* cultures maintained at the La Jolla laboratory have varied slightly over the years.

TABLE 3
Caloric and Ash Values

	n	% ash	(SD)	n	Calories/mg ash-free dry wt.	(SD)
Brachionus plicatilis						
Rotifers	7	7.8[1]	(2.0)	3	4.8	(0.3)
Tigriopus californicus						
Nauplii	2	16.2	(3.5)	3	5.8	(0.5)
Copepodites	2	17.0	(1.4)	2	6.0	(0.4)
Adults	2	7.3	(0.9)	2	5.9	(0.4)

[1]Theilacker and McMaster, 1971.

Copepod Dry Weight and Caloric Value

Naupliar dry weights ranged from 0.04 to 0.38 μg, and the weight increase was nearly proportional to the cube of the width. Copepodites weighed 2.5 to 3 times a nauplius of corresponding width (Table 2). Average dry weights given in Houde and Schekter (1981) for sieved samples of nauplii and copepodites were 0.15 μg per animal for the 53-110 μm fraction and 0.51 μg per animal for the 110-280 μm fraction. These width-weight estimates appear similar to ours (Table 2), but they are difficult to compare because the size component collected by sieving is not necessarily delimited by the sieve sizes used.

Assuming no between-culture differences, we averaged the caloric and ash values for each of the three copepod stages. The average caloric content of the organic fraction of *Tigriopus* adults and the young stages is similar, about 5.9 cal/mg (Table 4), and comparable to the 5.5 cal/mg reported for *Tigriopus* by Slobodkin and Richman (1961) and to the 5.6 cal/mg average for seven calanoid copepods given by Laurence (1976). (Laurence's values may be low because he used Formalin-preserved samples.) The value we

TABLE 4
Parameters (SE) for Nonlinear Equations ($y = a\,x^b$) for Estimating Rotifer or Copepod Dry Weight (y)[1] from Width, and Linear Equations ($y = a + bx$) for Estimating Dry Weight from Volume

Prey	n	Width[2] a	Width[2] b	Width[2] r^2	Volume[3] a	Volume[3] b	Volume[3] r^2
Brachionus plicatilis							
Rotifers	4	$1.4\times10^{-5}(2.1\times10^{-5})$	2.00 (0.29)	.970	0.029 (.033)	0.116 (.013)	.977
Tigriopus californicus							
Nauplii	4	$9.2\times10^{-8}(6.6\times10^{-8})$	2.90 (0.14)	.997	— —	— —	—
Nauplii and copepodites	6	— —	— —	—	0.020 (0.13)	0.189 (0.004)	.998

[1]y = dry weight (μg).
[2]x = width (μm).
[3]x = volume × 10^6 μm^3.

used to estimate caloric equivalents of individual prey (Table 2) is based on total tissue dry weight, i.e., organic plus ash weight, and is less for young stages because they have a relatively high inorganic (ash) content (Table 3). Young copepod stages have a higher ash content than the adults because the carapace imparts the greatest proportion of the ash weight, and the ratio of carapace weight to tissue weight is larger for small organisms. Our data also showed that females carrying egg sacs contained more ash than non-egg-bearing females and males (10.4% as compared to 7.3%), presumably because of the increased numbers of embryos in the sample. Razouls (1977) found the ash-free caloric value was higher for copepodites than adults, but her values are for preserved specimens and may not be comparable.

Regressions of dry weight (y) and body width (x) gave a good fit for both rotifer and nauplii data (Figure 1); estimates of the parameters for the dry weight and body width equations are given in Table 4. Although the dry weight (y) is subject to sampling error because it was estimated from a multiple regression (see Methods), its use to produce the dry weight and body width equations should be of little concern because the errors were negligible (Table 2). The equations given in Table 4 can be used to predict rotifer and naupliar dry weight from measurements of body width. We did not include an equation for the copepodites because we had only two points, but it is clear that at any given width a copepodite has a greater dry weight and caloric content than a rotifer, and a rotifer has a higher dry weight and caloric content than a nauplius.

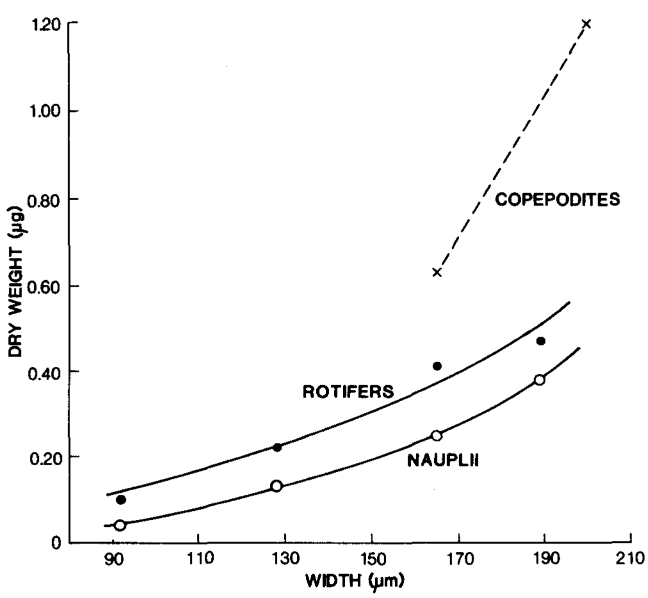

Figure 1. Relations between dry weight (µg) and body width (µm) for the rotifer *Brachionus plicatilis* and the copepod nauplii of *Tigriopus californicus*. Estimates of parameters for equations are in Table 2.

Rotifer and Copepod Volumes

Zooplankton biomass is often estimated using volume approximations (reviewed by Beers 1976), and the relations between copepod wet weight and volume are summarized by Pearre (1980). Because prey volumes are relatively easy to measure and have been used in feeding studies of marine fish (Sumida and Moser 1980), we related our dry weight measurements to estimated volumes and included equations for predicting dry weight for a given volume (Tables 2 and 4; Figure 2).

Copepod stages have a higher dry weight (and caloric content) than rotifers of the same volume (Figure 2). This may be due to unlike water content or to unlike densities, owing in part to dissimilarities in the weight or composition of their respective integuments. The average ash content of nauplii and copepodites was 16.2% and 17.0% as compared to rotifers, which had an assumed ash content of 7.8% (Table 3).

CONCLUSIONS

For any given width, rotifers have a higher caloric content than *Tigriopus* nauplii. First-feeding fish eating the smallest rotifers get twice as many calories per bite as they do eating *Tigriopus* nauplii of the same width (Table 2). Many first-feeding fish larvae select prey from the smallest size classes, yet older larvae eat prey belonging to all size classes. As a result, variation in estimated daily consumption by larval fish may be caused in part by the differences between the weight estimate for the average prey item and the actual weight of the item eaten. Clearly, an error in estimating food consumption can also result from

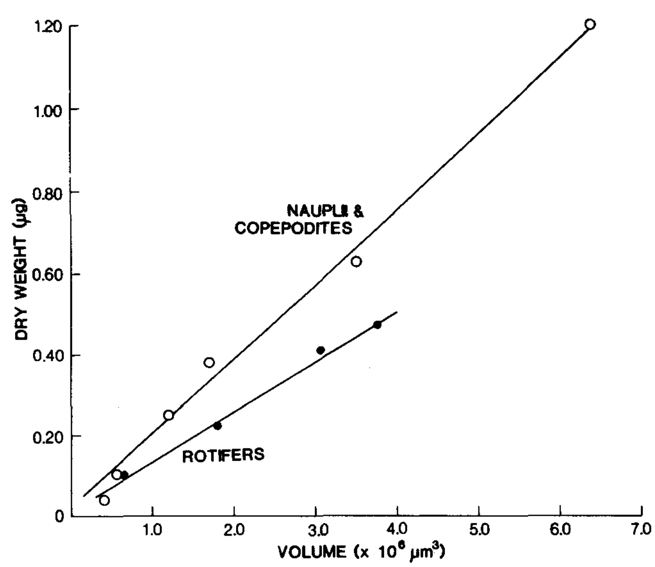

Figure 2. Relations between dry weight (µg) and volume of the rotifer *Brachionus plicatilis* and copepod *Tigriopus californicus*. Estimates of parameters for equations are in Table 2.

ignoring specific differences in caloric content among prey organisms.

Regressions of the width-weight measurements can be used to predict the dry weight of rotifers and nauplii of known width. The copepod width-weight model probably cannot be used for weights of calanoid life stages because calanoid nauplii are shaped more like an ellipsoid and thus would weigh more than dorsoventrally compressed harpacticoid nauplii of corresponding width. The copepod volume-weight model may be applicable to calanoid life stages.

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