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Rotifers as food in aquaculture

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Abstract

The rotifer *Brachionus plicatilis* (O.F. Muller) can be mass cultivated in large quantities and is an important live feed in aquaculture. This rotifer is commonly offered to larvae during the first 7-30 days of exogenous feeding. Variation in prey density affects larval fish feeding rates, rations, activity, evacuation time, growth rates and growth efficiencies. *B. plicatilis* can be supplied at the food concentrations required for meeting larval metabolic demands and yielding high survival rates. Live food may enhance the digestive processes of larval predators. A large range of genetically distinct *B. plicatilis* strains with a wide range of body size permit larval rearing of many fish species. Larvae are first fed on a small strain of rotifers, and as larvae increase in size, a larger strain of rotifers is introduced. Rotifers are regarded as living food capsules for transferring nutrients to fish larvae. These nutrients include highly unsaturated fatty acids (mainly 20 : 5 n-3 and 22 : 6 n-3) essential for survival of marine fish larvae. In addition, rotifers treated with antibiotics may promote higher survival rates. The possibility of preserving live rotifers at low temperatures or through their resting eggs has been investigated.

Introduction

The history of larval rearing of marine fish can clearly be divided into two distinct phases. Lack of adequate first feeds caused limited success in larval rearing of marine fish up to the sixties (Dannevig, 1897; Blaxter, 1968; Lasker *et al.*, 1970). This was before rotifers, primarily the species *Brachionus plicatilis*, were established as first larval feed. Most of the effort in this first phase was limited to small scale experiments of rearing larvae of different species for identification purposes or for fish stock assessment in fisheries (Houde & Palko, 1970). In these experiments, small prey organisms for the early larvae were ciliates, dinoflagellates, trochophores and wild plankton (Okamoto, 1969; Harada, 1970). The second phase started when rotifers were found to be suitable as first feed for marine fish larvae in Japan in the late sixties and early seventies. Rotifers became ubiquitous in all mass rearing trials after their successful use in the mass rearing of the red seabream (*Pagrus major*) in Japan (Fujita, 1973, 1979). The introduction of rotifers marked the first regular successes in the mass larval rearing of several marine species of economic value such as grey mullet (*Mugil cephalus*) (Nash *et al.*, 1974), sole (*Solea solea*) (Howell, 1973; Girin, 1974; Fuchs, 1978, 1982; Dendrinos & Thorpe, 1987), gilthead seabream

(*Sparus aurata*) (Person-Le Ruyet & Verillaud, 1980, 1981; Tandler & Helps, 1985), sea bass (*Dicentrarchus labrax*) (Barnabe, 1974; Girin, 1975), turbot (*Scophthalmus maximus*) (Kuhlmann *et al.*, 1981; Olsen & Minck, 1983; Witt *et al.*, 1984), and flounder (*Paralichthys olivaceus*) (Fukusho *et al.*, 1985), milkfish (*Chanos chanos*) (Liao *et al.*, 1979; Juario *et al.*, 1984) and others (Morales, 1983). These successes resulted from the development of rotifer culturing methods (Ito, 1960; Theilacker & McMaster, 1971; and reviews by Hirata, 1980; Fukusho, 1983; Hirano, 1987; Lubzens, 1987). Today, most marine fish are raised on basically the same methods, whereby *B. plicatilis* is provided as the first food during the first days of exogeneous feeding. The length of the period depends on the fish species.

Marine fish larvae are usually small at hatching (Theilacker & Dorsey, 1980; Kissil, 1984/85), and except for a few species, their size ranges between 2-7 mm. Rotifers offered to them must meet their nutritional requirements for optimization of growth and survival. These include: (1) the size; (2) the distribution and concentration of rotifers in the larval tanks; (3) the total amount available; (4) digestability and absorption; and (5) nutritional quality.

Size of rotifers

The size of the prey eaten by the fish larvae is a function of the larval mouth width. Within a fish species, mouth width is related to length, but it varies greatly between species (Arthur, 1976; Beyer, 1980; Hunter, 1980; Hunter & Kimbrell, 1980). Although mouth width limits the maximum prey size, in nature the mean diameter of the prey

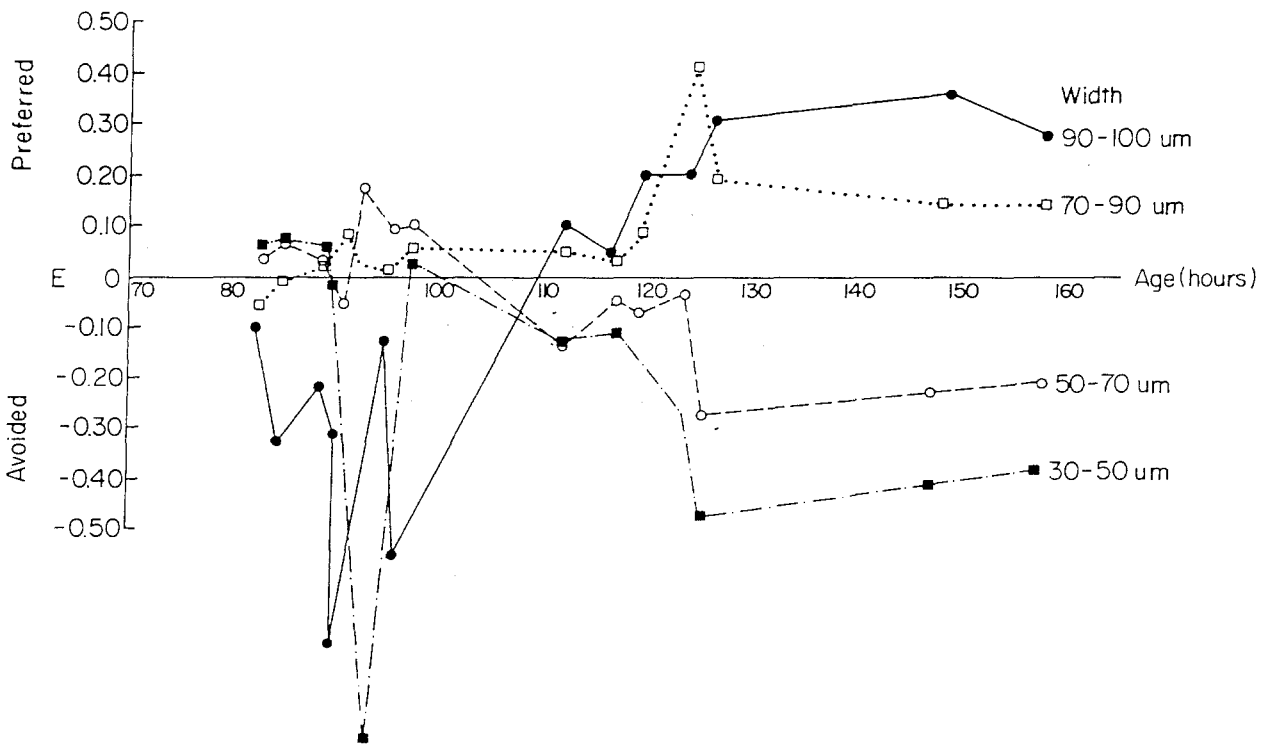

Fig. 1. Changes in the electivity indices (E) for larval sea bream (*Sparus aurata*) feeding on rotifers of various sizes as a function of larval age (redrawn from Helps [1982]). Electivity index (E) was calculated, based on Ivlev [1961], in the following way:

$$E = (r - p)/(r + p)$$

where r and p are the relative occurrence of a given rotifer size in populations exposed and unexposed to larval predation pressure, respectively.

consumed by fish larvae was only 38% of their mouth width (Hunter, 1980). There is a tendency for larvae in the sea to feed on progressively larger prey as they grow. In the laboratory it was found that the size of the prey fed to larvae must increase for larvae to grow at maximum rates (Lasker *et al.*, 1970; Hunter, 1980; Hunter & Kimbrell, 1980). Beyer & Laurence (1981) concluded that as larvae reach certain sizes, the energetic cost of each attack on prey exceeds the gain from ingesting smaller food particles. This size depends upon the larva's metabolic requirements, which are imposed both genetically and environmentally. It means that the larvae develop selection related to particle size both in natural populations and laboratory-reared species (Stepiens, 1976; Hunter, 1980).

The effect of age of gilthead seabream larvae (*Sparus aurata*) on their preference for rotifer strains of different sizes was examined in the laboratory (Helps, 1982). In this study, larvae showed a clear age effect on their feeding preference of different size rotifers (Fig. 1). Young larvae, up to 85 h after hatching, preferred small rotifers (30–70 μm in width) and avoided feeding on large rotifers (90–100 μm in width), while in older larvae (160 h) the pattern was reversed. The effect of rotifer size in long-term experiments was further tested, and it was found that the presence of small rotifers for the first 12 days of feeding of gilthead seabream larvae was associated with an improved growth rate (Tandler, pers. com.). This was especially pronounced in the size structure of the population of 32-day-old larvae. In these larvae, the percent of the population with a wet weight greater than 12 mg was 51.5 and 36, respectively, when small and large rotifers were offered as first food. In these studies, two genetically different

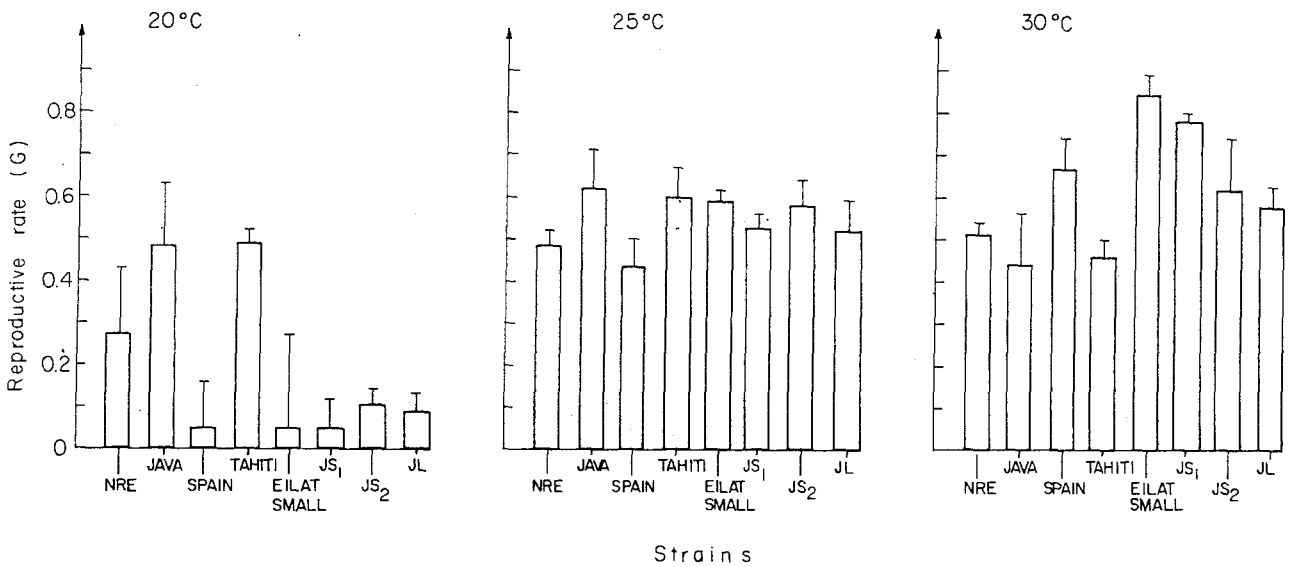

Fig. 2. The effect of temperature on the amictic reproductive rates of eight rotifer strains or clones cultured on *Nannochloris* sp. Reproductive rate was calculated from the equation:

$G = 1/T \ln N_t/N_o$,

where G_0 = experimental growth coefficient,
N_o = initial estimated population size,
N_t = final estimated population size,
T = time in days.

The mean values of five replicates and the standard deviations are presented by vertical bars.
Strains Java and Tahiti were obtained from INRA/IFREMER, France; strain Spain from Dr. E. Yufera; clones N.R.E. and Eilat Small from Israel; and clones JS_1, JS_2 and JL were hatched from resting eggs collected by Dr. M. Okauchi in Japan (from Lubzens *et al.*, in preparation).

size strains of rotifers were used. There is interest in these different size strains because they allow different larval stages to select prey of the most effective size (Fukusho, 1983; Korunuma & Fukusho, 1987).

Two methods of obtaining different sizes of rotifers have been considered; (1) sieving rotifer cultures through small size meshes which will allow the separation of small size rotifers from the larger ones; (2) culture of rotifer strains whose size is genetically determined. The first method does not guarantee full control over rotifer size. By sieving a rotifer culture, the small neonates will be separated from the adults, but within a short period they will eventually increase in size, while staying in the larval tanks. The culture of genetically different size rotifers offers a better solution (see Lubzens, 1987). However, this requires aquaculturists to maintain stocks of at least two rotifer strains in the hatchery.

Obtaining optimal mass cultures of different rotifer size strains may mean culturing them at different temperatures and offering them different types of food. For example, the small size strain in Japan (S-type) occurs in the summer when high temperatures prevail, while the large size rotifer strain (L-type) predominates in the winter (Fukusho & Iwamoto, 1980; Fukusho, 1983). Laboratory studies under controlled conditions of food and temperature show that the reproductive rate of rotifer strains is not always related to size (Fig. 2). Some of the small size strains (Java,

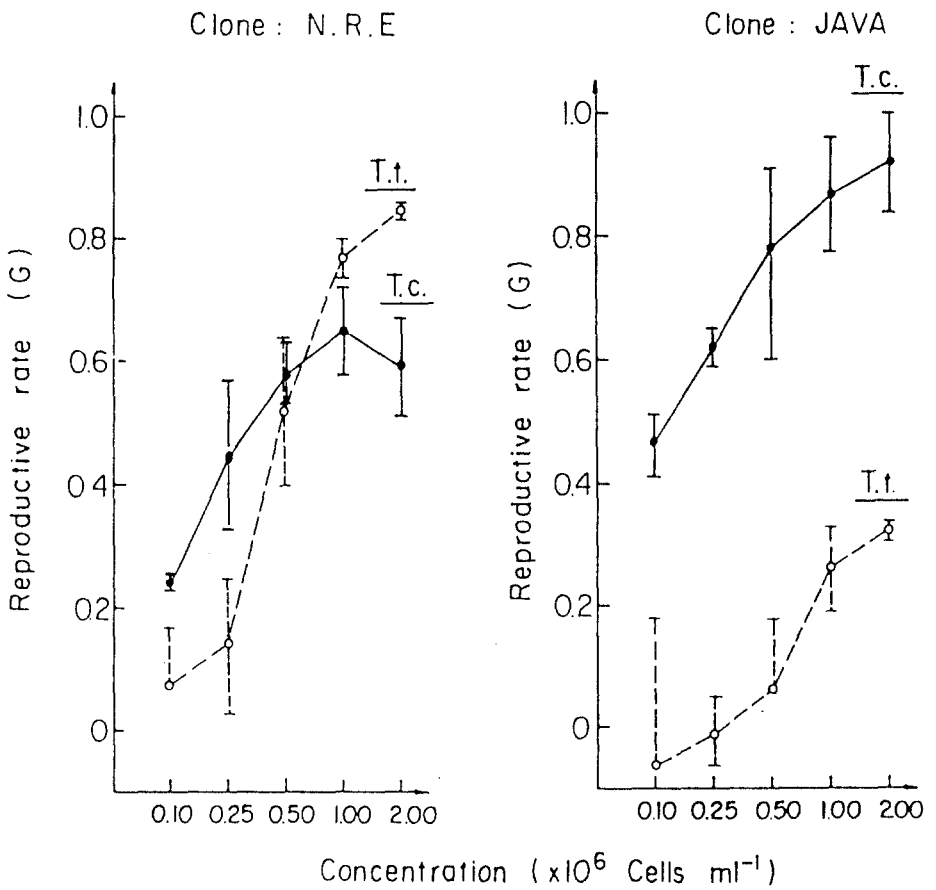

Fig. 3. The effect of two algae differing in size (*Tetraselmis tetrathele* and *T. chuii*) on the reproductive rates of large (clone N.R.E.) and small (strain Java) rotifers cultured at 25 °C. The average dimensions (in μm) of the algae were 15.3 × 8.5 × 10.5 and 12.0 × 6.5 × 6.0 for *T. tetrathele* and *T. chuii*, respectively. The average length and width (in μm) of the rotifers were 196.0 × 139.2 and 173.5 × 115.6 for clone N.R.E. and the Java strain, respectively (from Lubzens *et al.*, in preparation). The average reproductive rates and standard deviations were calculated as described in Fig. 2.

Tahiti) reproduce equally well at 20°, 25° or 30 °C while others (Spain, Eilat small, JS1 and JS2) clearly prefer high temperatures. Similarly, the large size strains (N.R.E. and JL) higher reproductive rates were found at 25 °C or 30° than those at 20 °C. Furthermore, a positive correlation was found between body size and maximum size of particles ingested (Hino & Hirano, 1980). It is likely that large size algae (e.g. *Tetraselmis tetrathele*), which are currently popular in feeding rotifers, will not easily be ingested by small rotifers. Small rotifers cultured on such algae will probably have low reproductive rates (Fig. 3). Consequently, choosing the appropriate rotifer strains to be mass cultured will depend on the environmental conditions prevailing at the hatchery and the size of larval predators. Moreover, in offering different rotifer size strains, their dry weight must be taken into account in evaluating the number of rotifers required by the predatory larvae (see below).

The distribution and concentration of rotifers in larval tanks

The distribution of the rotifers in the water column of larval tanks depends mainly on the salinity and to a lesser extent on water quality. Exposing rotifers to sudden changes in salinity will result in their adhesion to the bottom or sides of containers (Gatesoupe & Luquet, 1981; Lubzens, 1987), making them unavailable to the larvae. Other factors affecting the distribution of rotifers are oxygen and ammonia levels which affect the swimming speed (Epp & Winston, 1978; Snell *et al.*, 1987) and the type of food offered to rotifers.

The nutrition of fish larvae depends primarily on the probability of encounter between the food and the larva as well as its suitability in terms of size and nutrient composition. The probability of encounter between the larva and the rotifer depends on concentration. In turn, the perception of food organisms affects larval swimming behavior (Hunter, 1980). Several studies showed a direct correlation between food concentration and larval survival (Theilacker & Dorsey, 1980).

The optimal food concentration depends on the life stage and temperature. First feeding larvae have relatively slow swimming speeds (Fukuhara, 1983) and low capture success at onset of feeding (2-10%) (Hunter, 1980), so they may require a high rotifer concentration. This concentration could be reduced up to the stage where the larvae may demand higher quantities in order to meet energetic requirements. Up to a rotifer concentration of 10 ml^{-1}, a direct relationship between survival, growth and food concentration was shown in gilthead seabream (Tandler & Sherman, 1981; Peguin, 1984), and in bream (*Archosargus rhomboidalis*) (Dowd & Houde, 1980). Beyond this concentration, a sharp drop in both growth and survival of seabream larvae was observed (Tandler & Sherman, 1981; Peguin, 1984). These results, however, were not related to an increase in the metabolite concentration (NH_3-NH_{4+}) in the water body. In a study in which rotifers, labelled with ^{14}C, were offered to seabream larvae, at concentrations of 10 and 40 ml^{-1}, it was shown that feeding rate of these larvae was directly correlated to rotifer concentration (Tandler & Mason, 1984). Therefore, it can be proposed that the negative effect of elevated rotifer concentrations on growth were associated with reduced efficiency of the digestive process at high rotifer concentrations. Similar observations were made by Boehlert & Yoklavich (1984), who found that the assimilation efficiency of herring larvae (*Clupea harengus*) was negatively and logarithmically correlated to rotifer concentrations between 1 and 10 ml^{-1}.

Almost no information is available on the effect of larval density in the culture tanks on rotifer concentration. Okamoto (1969) showed that survival of larvae is clearly related to larval stocking density, but it is not known whether this is related also to the shortage in food which may occur at high larval densities.

Marine fish larvae depend primarily on vision to find their food, as a result of the pure cone retina found in the majority of marine larvae (Blaxter & Staines, 1970; Blaxter, 1975). Photoperiod, light intensity and color of background of rearing containers are therefore of paramount importance for their hunting success.

The effect of photoperiod in combination with rotifer concentration is intimately related to the probability of encounter between the larva and its food (Dowd & Houde, 1980; Peguin, 1984). In both studies it was found that at low rotifer concentrations, there was a direct relationship between the duration of the photoperiod and growth. At high rotifer concentrations of 25 ml^{-1} in gilthead seabream (Peguin, 1984) and 100 to 500 l^{-1} in seabream (Dowd & Houde, 1980), there was a mid-range photoperiod which supported best growth, beyond which a further increase was associated with no improvement in the former and with reduced growth rates in the latter species. A similar response of growth to a mid-range photoperiod of 18 h was reported by Barahona-Fernandes (1979) for sea bass (*Dicentrarchus labrax*). On the other hand, Fuchs (1978) obtained best larval growth in sole (*Solea solea*) under continuous illumination. Survival of both gilthead seabream (Peguin, 1984) and seabream (Dowd & Houde, 1980) peaked at mid-range photoperiods of 15 and 13 h, respectively. In seabream, the effect of photoperiod was independent of rotifer concentration, while in gilthead seabream, a direct correlation between photoperiod duration and survival was observed only at low food concentrations of 1.0 ml^{-1}.

The number and concentration of rotifers supplied to fish cultures is expected to vary with temperature. Temperature will affect several processes in the growth and survival of larvae, among them metabolic rate and swimming activity (Theilacker & Dorsey, 1980). This will result in higher predation and energy (food) requirements.

Availability of rotifers

The large quantities of rotifers required for raising marine fish larvae (40 000–173 000 per surviving larva) (Okauchi *et al.*, 1980 Kafuku & Ikenoue, 1983) are of constant concern to the aquaculturist. The development of high density mass culture techniques (Hirata, 1980; Lubzens, 1987; Yamasaki *et al.*, 1988) allows for constant production of rotifers, but does not insure against sudden shortages in supply as a result of unforeseen collapses. Preventive measures may be taken, to some extent, by routine daily monitoring of swimming speed, proportion of eggs (Snell *et al.*, 1987), and physical parameters (pH, oxygen level, etc.). Preserved rotifers could be helpful in the management of supply and demand in the hatchery. In this respect, the possible use of artificially produced rotifer resting eggs has been evaluated in recent years (Lubzens, 1981, 1989). It was found to be too expensive for routine use, in the way that *Artemia* cysts are used today in aquaculture. In extreme cases, these eggs could be used to initiate small-scale new cultures. Preservation of live rotifers at low temperatures has been examined recently (Berghahn *et al.*, 1989; Lubzens, 1989; Lubzens *et al.*, 1989). This method would permit the preservation of different strains or clones, including those that do not produce resting eggs. In those cases where *B. plicatilis* is abundant, it could also be used for culturing of freshwater fish larvae, e.g. ayu (*Plecoglossus altivelis*) or cyprinids (*Cyprinus carpio*) (Kanazawa *et al.*, 1982; Lubzens *et al.*, 1987).

Ingestion, digestion and conversion rates of rotifers by fish larvae

Ingestion

As already mentioned above, fish larvae tend to increase both the quantity and the size range of particles upon which they feed throughout their ontogeny. The increase in the number of food items ingested daily by fish larvae is an exponential function of larval age (Stepiens, 1976; Buckley & Dillmann, 1982) or length (Okauchi *et al.*, 1980; Barahona-Fernandes & Conan, 1981). In absolute terms, larval daily rations increase as a linear function of their weight (Laurence, 1977; Houde & Schekter, 1983; Klumpp & von-Westernhagen, 1986; Minkoff, 1987). The numbers of rotifers consumed daily (Rn) as a function of larval length (L) has been determined for porgy (*Acanthopagrus schlegeli*) as $Rn = 2.43\ L^{3.05}$ (Okauchi *et al.*, 1980) and red sea

bream (*Pagrus major*) as $Rn = 0.39\ L^{3.67}$ (Fujita, 1979). Likewise, the dry weight of rotifers consumed daily, *Rw* (daily ration) as a function of larval dry weight (*Wt*, in mg) has been evaluated for the blenny (*Blennius pavo*) as

$$Rw = 314.5\ Wt + 26.3$$

(Klumpp & von-Westernhagen, 1986), herring (*Clupea harengus*) as

$$Rw = 0.13\ Wt + 49.67$$

and turbot (*Scophthalmus maximus*) as

$$Rw = 0.43\ Wt + 38.45$$

(Minkoff, 1987). Concomitant to the increase in feeding rates, the growing larvae tend to require larger food particles. This factor has led to the use of different rotifer strain sizes in rearing of fish larvae (see above).

Conversion

Rotifers were found to contain acid proteinase, alkaline proteinase and two kinds of alkaline proteases (Hara *et al.*, 1979a, b, 1984). All the current data show that larvae at first feeding seem to possess the necessary complement of enzymes for digesting their prey (e.g. Govoni *et al.*, 1986).

The assimilation of ingested rotifers, or any other food organism, by fish larvae has been shown to be very rapid. ^{14}C originating from labeled rotifers has been found to be respired by fish larvae 3 h following their ingestion (Govoni *et al.*, 1982).

The gross growth efficiency, which is the proportion of the ingested food invested in growth (Ki) (Brett & Groves, 1979), has been evaluated for larvae feeding on rotifers; for herring at 20–60%, turbot 20–40% (Minkoff, 1987) and blenny 50–56% (Klumpp & von-Westernhagen, 1986). The values obtained for the first two species are similar to values reported for other fish species feeding on other types of prey (Govoni *et al.*, 1986; Klumpp & von-Westernhagen, 1986).

One factor which influences the assimilation efficiency in the early larval stages is that of food density. As already discussed before, larvae, being 'number maximizers', tend to consume prey in relation to availability rather than according to satiation. Therefore, at high prey densities they will tend to consume food at a rate which decreases its residence time in the gut with a concomitant reduction in the assimilated efficiency (Boehlert & Yoklavich, 1984).

Nutrition

The current knowledge pertaining to marine fish larval nutrition has been derived through the analysis of the performance of the larvae while feeding on different rotifers 'enriched' with various nutrients and through testing of formulated inert diets. It was found that rotifers cultivated on baker's yeast alone could not support larval growth or survival. The failure of these rotifers was attributed to their lack of the long chain highly unsaturated fatty acids (HUFA) on the n-3 series, mainly 18 : 3 n-3, 20 : 5 n-3 and 22 : 6 n-3 (Owen *et al.*, 1975; Yone & Fujii, 1975; Cowey *et al.*, 1976; Fujii & Yone, 1976; Gatesoupe *et al.*, 1977; Watanabe *et al.*, 1983; Dendrinos & Thorpe, 1987). These fatty acids are found in some of the algae of marine origin. This has led to an extensive search for species of algae which are most suitable for enrichment of rotifers. From a practical aspect, using yeast to raise rotifers instead of algae reduces greatly the production costs in the hatchery. Furthermore, replacing the algal enrichment step with inert rotifer diets will assist in curtailing the production price of nutritionally adequate rotifers.

Rotifer biochemical composition in relation to larval requirements

The nutritional value of rotifers to larvae depends on their dry weight, caloric content and biochemical composition. The dry weight of a single rotifer, excluding eggs, as measured by Doohan (1973), Tandler & Mason (1984), Theilacker &

Kimball (1984), Yufera & Pascual (1984) and Minkoff (1987), ranges between 120-360 ng/ind, depending on the nutritional state and body size. Scott & Baynes (1979) estimated that during periods of high growth rates and ample food supplies, the rotifer's weight could increase up to 620 ng/ind. Furthermore, the latter authors have also demonstrated that at 24-26 °C, in the absence of food, the rotifer loses daily between 18-26% of its body weight. The influence of the dietary condition on the rotifer's dry weight has also been noted by Minkoff (1987), who found that rotifers raised on baker's yeast alone increased their dry weight by 12-23% following prolonged (48 h) enrichments with algae. Conversely, rotifers lost 30% of their body weight following a 24 h starvation period at 20 °C. The rapid loss of organic material from rotifers which are deprived of food is generally perceived as one of the main factors causing poor growth and high mortalities in fish larval cultures. This is probably the reason for the use of the 'green water' by most larval culturists, which maintains the rotifers in a healthy nutritious state.

The other prominent factor influencing the rotifer's weight is that of the animal's size, which, as pointed out previously, is mainly strain-dependent. Theilacker & Kimball (1984) have described the relationship between the width (X, in μm) and dry weight (Y, in ng) of *B. plicatilis* to be $Y = 1.4 \times 10^{-5} X^2$.

Direct caloric measurements show that the ash-free caloric value of a rotifer ranges from 4.8 cal/mg (Theilacker & Kimball, 1984) to 6.46 cal/mg (Minkoff, 1987). Again, the value per individual will depend on both the size and nutritious state of the organism. Moreover, it was reported (Kentouri & Divanach, 1982; Minkoff, 1987), and also found in *Sparus aurata* cultures (Tandler, pers. com.), that rotifer eggs and the rotifer lorica are not digested by the fish larvae. Therefore, it is essential to correct for egg/female ratios in the estimates made of the nutritional requirements of larvae (Minkoff, 1987).

Most researchers have noted that the species of algae or yeast had only minor effects on the biochemical composition of rotifers fed on them, and the values reported fall within the range of the experimental error. Both Scott & Baynes (1978) & Minkoff (1987) have found protein in the range of 50-58% of the rotifer's dry weight. Watanabe *et al.* (1983) have set protein levels at 65%, while Dendrinos & Thorpe (1987) found these levels to be 34-36%. Contrary to this, Ben-Amotz *et al.* (1987) have reported a much wider range of crude protein from 28-51%. Lipids usually range from 9-16% of the dry weight (Scott & Baynes, 1978; Minkoff, 1987), and up to 23.5% and 28% (Watanabe *et al.*, 1983; Dendrinos & Thorpe, 1987) (Table 1). One observation seems to be consistent throughout the literature: rotifers fed only on baker's yeast are poorer in their total lipid content than their counterparts that had either been cultivated only on algae or had received some algae in their diet. This low level of lipids can be elevated by prolonged feeding on algae (Minkoff, 1987) or by feeding rotifers with lipid-enriched yeasts (Dendrinos & Thorpe, 1987).

Inasmuch as marine fish require a diet containing 40-60% protein (Castell *et al.*, 1986) and 13-16% lipid, there is no doubt that rotifers fulfill these requirements. However, for the rapidly developing larval stages, it is probable that the balance of dietary amino acids, fatty acids, vitamins and minerals determines the success of organogenesis rather than the gross composition of the diet.

The amino acid profiles of rotifers enriched on a variety of marine algae or yeast have been determined by a number of researchers (Watanabe *et al.*, 1983; Dendrinos & Thorpe, 1987; Minkoff, 1987; Rezeq & James, 1987). These analyses show no significant differences in rotifer amino acid profiles and therefore fail to explain the advantage of certain algae used for rotifer enrichment. Furthermore, the amino acid profiles of rotifers do not seem to deviate significantly from those of other zooplanktonic organisms either cultured or of wild origin, which are recognized as being beneficial for growth of larvae (Castell *et al.*, 1986).

Undoubtedly, the most significant dietary factors to influence the growth and survival of marine fish larvae are the highly unsaturated fatty acids

Table 1. Proximate dry weight values of *B. plicatilis* cultured on different diets

Diet	Protein %	Lipid %	Carbo-hydrate %	Ash %	Authors
Isochrysis galbana	36.3	21.1	–	–	Dendrinos & Thorpe, 1987
Nannochloris oculata	34.5	21.4	–	–	Dendrinos & Thorpe, 1987
Saccharomysis cerevisiae	41.6	14	–	–	Dendrinos & Thorpe, 1987
Saccharomysis cerevisiae L4	33.17	24	–	–	Dendrinos & Thorpe, 1987
Saccharomysis cerevisiae L5	42.3	28.5	–	–	Dendrinos & Thorpe, 1987
Chaetoceros gracilis	32	20.1	44.9	–	Ben-Amotz *et al.*, 1987
Chlorella stigmatophora	28.1	7.4	10	–	Ben-Amotz *et al.*, 1987
Isochrysis galbana	29.1	11.1	7	–	Ben-Amotz *et al.*, 1987
Nannochloropsis salina	28	16.4	24.1	–	Ben-Amotz *et al.*, 1987
Phaeodactylum tricornutum	54.4	8.9	13.5	–	Ben-Amotz *et al.*, 1987
Yeast	55.4	4.5	28	–	Ben-Amotz *et al.*, 1987
Yeast	65–67	15–21	–	10	Watanabe *et al.*, 1983
Chlorella minutissima	58–72	21–31	–	4–9	Watanabe *et al.*, 1983
Chlorella + yeast	63–67	20–22	–	4–6	Watanabe *et al.*, 1983
Nannochloropsis oculata	50–53	9–13	–	–	Minkoff, 1987
Isochrysis galbana	50–54	10–12	–	–	Minkoff, 1987
Yeast	53–57	6–9	–	–	Minkoff, 1987

(HUFA) of the n-3 series. These fatty acids most likely form a part of the larvae's cellular membranes and therefore are crucial in determining the rates of enzymatic processes taking place at these sites. It is well established that in rotifers, the fatty acid profile is chiefly determined by diet (Scott & Middleton, 1979; Watanabe *et al.*, 1983) and that only minor alterations in the fatty acid composition can be achieved through *de novo* synthesis by the rotifer itself (Lubzens *et al.*, 1985). These lipids are digested by the rotifer and incorporated into its cellular phospholipids (Lubzens *et al.*, 1985). Rotifers which are raised on baker's yeast alone are inadequate as food for larvae due to their lack of these fatty acids. Therefore, rotifer enrichment procedures were developed to insure the ready supply of nutrients required by the developing larva. The enrichment regimes are based on the immersion of rotifers for 8-24 hours in a medium rich in essential nutrients, especially n-3 HUFA. The enrichment medium may contain one of the following constituents: (1) different algal species (Kitajima *et al.*, 1979), for example: *Isochrysis galbana* (Howell, 1979; Tandler & Helps, 1985); *Nannochloropsis* sp. and *Tetraselmis* sp. (Fukusho *et al.*, 1984); (2) a special yeast enriched with (n-3)HUFA (Kitajima *et al.*, 1980). (3) emulsions which are based on (n-3)HUFA rich oil from marine fish or cuttlefish extracts (Watanabe *et al.*, 1983). (4) a dry diet for rotifers which both supports their culture and improves their quality as food for sea bass larvae (Gatesoupe & Luquet, 1981; Gatesoupe & Robin, 1982). (5) microcapsules (Walford & Lam, 1987).

The dietary HUFA requirements for most marine fish larvae have not yet been determined in a way that will differentiate between a need for either 20 : 5 n-3 or 22 : 6 n-3 or both. Red sea bream larvae have very similar growth rates when fed on rotifers containing the C : 20 HUFA incorporated from *Chlorella*, as on rotifers containing both the C : 20 and C : 22 HUFA incorporated from yeasts cultured in the presence of marine lipids (Kitajima *et al.*, 1980). The ayu, *Plecoglossus altivelis*, which spends most of its early life history in fresh water, has a dietary requirement for 18 : 3 n-3 (Watanabe *et al.*, 1983) which is very similar to what is known for salmonids (Kanazawa, 1985). However, the ayu can benefit from the input of both the C : 20 and C : 22 FA into its diet (Oka *et al.*, 1980). A recent attempt to differentiate between the requirements for C : 20

and C : 22 FA in a range of larvae suggests that only turbot have an essential fatty acid (EFA) requirement for both C : 20 and C : 22 HUFA, while herring and plaice thrive equally well when only the C : 20 HUFA or both the C : 20 and the C : 22 are present in the diet (Minkoff, 1987).

Can rotifers be replaced by inert diets?

At present the aquaculturist views the rotifer mainly as a 'living food capsule' through which nutrients essential to the fish larva can be transferred (Watanabe *et al.*, 1983). Special rotifer feeding techniques were developed for their 'enrichment' with various essential nutrients. Lately, larvae are being offered rotifers which have been enriched with antibiotics. Gatesoupe (1982), for example, found that larval turbot given rotifers which were previously immersed in a 48 mg l^{-1} Tribrissen for 0.5 hr grew faster and survived better than the untreated control.

The general dependence on live feeds, mainly rotifers, is a constraint as far as dietary manipulation of feed body composition is concerned. Therefore, efforts were recently made by various laboratories to develop inert microdiets in which the composition is easy to alter. At present, however, microdiets cannot completely replace rotifers in first feeding marine fish larvae. In most of the reports, microdiets are offered to marine fish larvae as a partial replacement only 8-10 days after hatching (Kanazawa *et al.*, 1982; Kanazawa, 1985). Kissil (1984/85) correlated the larval size of first feeding larvae of 10 species and found that the minimum length of fish reported to feed on formulated diets is 7 mm. This suggests that the minimum age/size recommended for successful use of inert diets is associated with the stage of development of the digestive system. A similar conclusion was reached by Dabrowski (1984), who proposed that the advantage of live feeds results from the presence of digestive enzymes, and the activation of zymogens in the developing larval digestive tract.

Culture of crustacean larvae important in aquaculture with *B. plicatilis*

Several reports show that rotifers may fill the gap between the period phytoplankton and *Artemia* are offered to developing shrimp larvae (Mock *et al.*, 1980). Rotifers are consumed by zoea stages 2-3 of *Penaeus japonicus* (Hirata *et al.*, 1985), *P. kerathurus* (Yufera *et al.*, 1984), and *P. semisulcatus* (Watanabe, 1980; Samocha *et al.*, 1988). *P. indicus* has been shown to consume rotifers as early as Z 1 (Emmerson, 1984). It has been recently challenged by Samocha *et al.* (1988) that since rotifers are less adequate and more expensive than *Artemia* (Tandler, 1984/85), they are not essential in culturing commercially important crustaceans, but if available may replace *Artemia* for short periods (Yamasaki & Hirata, 1982).

Summary and conclusions

Rotifers of the species *Brachionus plicatilis* were found to be primarily an essential live food source for larvae of several species of marine fish and to a lesser extent for larvae of shrimp or crabs. Rotifers possess several characteristics that make them attractive as live food in mariculture: (1) They are relatively small, ranging in size from 0.06–1.00 mm, depending on zoogeographical strain and stage of development; (2) They are slow swimmers, maintaining their position in the water column; (3) They can be cultured at high densities; (4) They reproduce rapidly, making them available in large quantities within a relatively short period; (5) They can be enriched with fatty acids or antibiotics which are required for growth and survival of larvae.

The development of standardized mass culture techniques insures, to some extent, an adequate supply of the large numbers of rotifers required to raise commercially important fish species. The number of rotifers required depends on the size of the rotifer strain and the duration it is supplied in the fish larval tanks. The temperature prevailing in the culture site, the salinity of the water, and the

food offered to rotifers will determine to a large extent the rotifer strain chosen for cultivation. Choosing the appropriate strain is important for obtaining high reproductive rates, thus reducing the rotifer 'standing stock' in the hatchery.

In order to ensure adequate amounts of essential lipids, rotifers must be enriched with either an appropriate alga, emulsified fresh oils or inert particles such as microcapsules. The nutritional quality of the rotifers, which depends on their state of satiation, is maintained in the presence of algae in the larval tanks. The duration of providing live food to larval tanks is constantly reduced with the concomitant development of inert foods. The possible future development of preserved live rotifers may improve the management of supply and demand of rotifers in the hatchery and may open the possibility for using *B. plicatilis* as food for raising problematic freshwater fish species.

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