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Co-feeding microparticulate diets with algae: toward eliminating the need of zooplankton at first feeding in larval red drum (*Sciaenops ocellatus*)

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Abstract

The effect of adding algae to the culture water used to rear red drum larvae was evaluated in terms of growth, survival and digestive enzyme activity. Red drum larvae were subjected to one of the following dietary regimes from first feeding (day 3 post-hatch) to day 14: (1) zooplankton supplemented with algae (L-A), (2) zooplankton without algae (L-NA), (3) a microparticulate diet with algae (M-A) and (4) the microparticulate diet alone (M-NA). The presence of algae in the rearing tanks improved growth of red drum larvae for both types of feeds. Growth was significantly higher ($P < 0.05$) in larvae reared in the presence of algae (L-A and M-A) than in larvae raised in the corresponding treatments without algae (L-NA and M-NA). Red drum larvae raised on the microparticulate diet and algae (M-A) grew as well as the zooplankton treatment with no algae (L-NA), and were not significantly different from the L-A treatment. The larvae fed the microparticulate diet in the absence of algae (M-NA) were significantly smaller than the other three treatments. These results were consistent for two separate feeding trials. Final survival was highly variable in all treatments; nevertheless, mean final survival values were 30% higher in treatment L-A compared to L-NA (14.1 and 10.1%, respectively) and 42% higher in M-A than for M-NA (6.2 and 4.0%, respectively). Significantly higher trypsin and aminopeptidase activity was observed in the presence of algae, which may have influenced the digestion of the diet. Our results

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demonstrate that red drum larvae may be raised on a microparticulate diet from first feeding without the use of zooplankton.

Keywords: Marine fish larvae; Microparticulate diets; Algae; First feeding; Digestive enzymes

1. Introduction

One of the limitations of larval fish nutrition studies is the dependence on live feeds such as rotifers and *Artemia*, which cannot be manipulated as desired, in order to determine nutritional requirements of the larvae. Nutrients present in zooplankton have either high ''background'' concentrations of the nutrients of interest (i.e., ascorbic acid, Merchie et al., 1997) or their quantities and ratios cannot be modified to a desired level as a result of the highly conservative nature of some nutrients, such as the polar lipid classes (Rainuzzo et al., 1994). Thus, it would be ideal to produce a microparticulate (MP) diet to replace the use of zooplankton. Unfortunately, despite considerable advances, an adequate replacement for live feeds from first feeding has yet to be developed.

Ingestion rates of MP diets are often lower than those of live food during the first days of feeding, which may limit the availability of nutrients for proper growth and development (Lauff and Hoffer, 1984; Kolkovski et al., 1993). Previous research at our laboratory has shown that feeding red drum larvae a MP diet from first feeding does not support adequate growth and survival (Holt, 1993; Lazo et al., 2000). From visual observations, we suspect poor ingestion rates (i.e., the volume of gut contents appears to be smaller in larvae fed MP diets than those fed zooplankton). In addition, the MP diet may be poorly digested and probably fails to meet the nutritional requirements of red drum larvae.

The addition of algae to the rearing tanks of marine fish larvae (green water culture) has been shown to enhance growth and survival as well as the quality of the fry (see review by Reitain et al., 1997). One of the beneficial effects attributed to adding algae is an increase in ingestion rates of food by marine fish larvae (Naas et al., 1992). In addition, the presence of algae in rearing tanks of European sea bass larvae has been shown to increase digestive enzyme secretion (Cahu and Zambonino-Infante, 1998), but this was not evaluated with an MP diet as the sole feed at the onset of feeding, a more crucial developmental stage.

The aim of this experiment was to evaluate the effects of adding algae to the rearing tanks of red drum larvae fed only a microparticulate diet from the onset of first feeding on growth, survival and activity of two digestive enzymes; trypsin and aminopeptidase. The effects of algal additions were evaluated for red drum larvae raised on zooplankton or a microparticulate diet from first feeding.

2. Materials and methods

2.1. Feeding trials

Two separate growth trials were performed to evaluate the effects of adding algae to the rearing tanks. Larvae were raised utilizing a modified version of the rearing protocol

described by Holt (1993). Following initiation of exogenous feeding (day 3), four replicate tanks were subjected to one of the following dietary regimes using a 2×2 factorial design: (1) zooplankton supplemented with algae (L-A), (2) zooplankton without algae (L-NA), (3) microparticulate diet with algae (M-A) and (4) microparticulate diet alone (M-NA).

Red drum (*Sciaenops ocellatus*) eggs were obtained from broodstock spawned in our laboratory under controlled temperature and photoperiod (Arnold, 1988). Following hatching (day 1), larvae were stocked to individual 150-l tanks at a density of 12 larvae per liter. Photoperiod was set on a 12:12 h light/dark cycle throughout the study. Temperature was kept near 26 ± 1°C, dissolved oxygen above 6.0 mg l^{-1} and salinity 32 ± 1 g l^{-1}. Total ammonia-nitrogen and nitrite-nitrogen levels were monitored twice weekly during the first trial using photometric methods (Spotte, 1979). In addition, pH was measured twice per week.

The microparticulate diet (MP) used was a microbound diet (Fry Feed Kyowa, Kyowa Hakko Kogyo, Tokyo, Japan). The proximate composition of the diet is presented in Table 1. The feeding protocol for the MP diet and live prey (rotifers and *Artemia*) was the same as previously described by Lazo et al. (2000). The species of algae added to the culture tanks was the marine species *Isochrysis galbana*. Concentrations were maintained between 40,000 and 60,000 cells ml^{-1} throughout the study.

2.1.1. Sampling and dissection

For growth and enzyme activity determinations, larvae were collected prior to morning feeding on days 3, 4, 6, 8, 10, and 14. Following collection, larvae were maintained in beakers without food for at least 1 h to allow any remaining food in the gut to be assimilated or excreted. At least 24 larvae from each tank were subsequently anesthetized with 0.1% tricaine methanesulfonate (MS-222) and standard length measured with a stereomicroscope using a digitizing tablet and Sigma Scan software (Ver. 3.90, Jandel Scientific, CA). Digestive organs of 16 individual larvae per tank were

Table 1
Proximate composition of the microparticulate diet (Kyowa Fry Feed)[a]

Nutrient	Kyowa (g kg^{-1} dry weight)
Moisture	53.0
Crude protein	511.0
Crude lipid	248.0
Fatty acids	
$\Sigma(n-3)$ HUFA[b]	43.0
$\Sigma(n-6)$ PUFA[c]	12.0
DHA:EPA[d]	1.1
Energy[e]	24.3

[a] From Brinkmeyer and Holt (1998).
[b] Higly unsaturated fatty acids.
[c] Polyunsaturated fatty acids.
[d] Docosahexaeonic acid (DHA, 22:6*n*-3): eicosapentaeonic acid (EPA, 20:5*n*-3).
[e] Gross energy (J kg^{-1} diet).

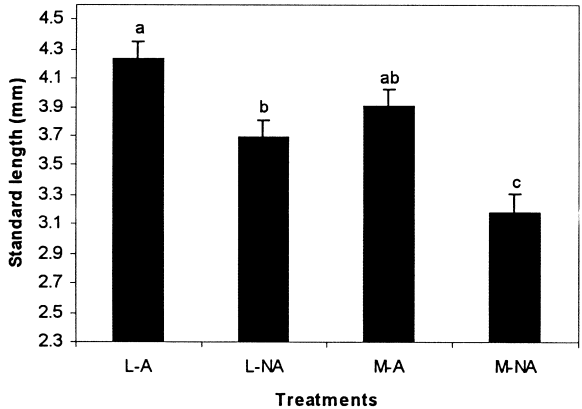

Fig. 1. Final standard length of red drum larvae fed the various dietary regimes. Error bars represent pooled standard error ($n = 8$). L-A and L-NA represent larvae fed zooplankton with and without algae, respectively. M-A and M-NA represent larvae fed microparticulate diet with and without algae, respectively.

dissected and removed (except for day 3 larvae, due to their small size). A cut was made at the boundary between the esophagus and the midgut and the whole intestinal segment (midgut and hindgut), including the diffuse pancreas embedded in its outer surface, was removed and immediately frozen. Dissections were done under a dissecting microscope on a glass slide maintained at 0°C. Guts from eight larvae were pooled into one sample for subsequent digestive enzyme activity measurements.

2.2. Digestive enzyme assays

Frozen guts were homogenized in ice-cold homogenization buffer (20 mM Tris–HCl, pH 7.5) with a tissue grinder. Four replicates per treatment (where one replicate

Table 2
Two-way analysis of variance of the effect of age (days after hatching) and algae on enzyme activity in red drum larvae

	$P \leq$		
	Age	Algae	Age × Algae
Total activity			
Trypsin	0.001	0.010	0.050
Aminopeptidase	0.001	0.001	0.001
Specific acitivity			
Trypsin	0.050	0.442	0.872
Aminopeptidase	0.324	0.010	0.730

consisted of eight guts from one tank) were assayed in duplicate for enzyme activity and protein content. Trypsin-like enzyme activity was measured in centrifuged homogenates (30 min at $1700 \times g$) using BAPNA (10 mM in DMSO) as substrate in 50 mM Tris–HCl buffer (10 mM $CaCl_2$, pH 8.2) following the method of Erlanger et al. (1961). One unit of enzyme activity was defined as 1 μmol of *p*-nitroaniline released per min, using an extinction coefficient of 8800 M^{-1} cm^{-1}. Aminopeptidase activity was estimated from uncentrifuged homogenates using L-leucine-*p*-nitroanilide, (1.2 mM in DMSO) as substrate in 50 mM Tris–HCl buffer (pH 8.0) following the method of Appel (1974). One unit of enzyme activity was defined as 1 μmol of *p*-nitroaniline released per minute, using an extinction coefficient of 10,500 M^{-1} cm^{-1}. Measurements of soluble protein content in each separated fraction (i.e., uncentrifuged and centrifuged) were completed using the bicinchoninic acid protein assay kit (Sigma, Cat. No. BCA-1).

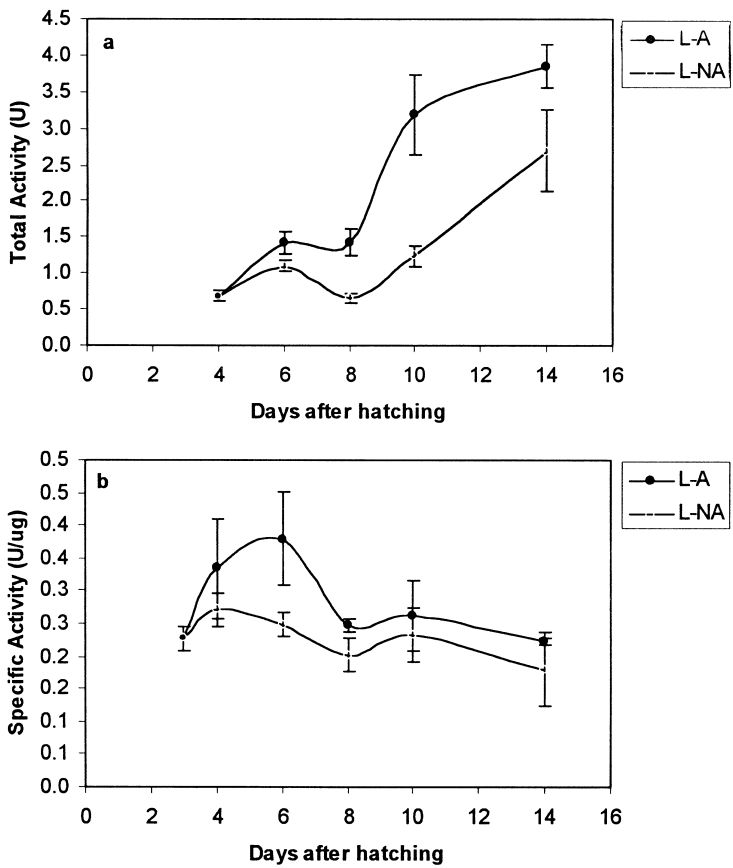

Fig. 2. Relationship between (a) total trypsin activity and age, and (b) specific trypsin activity and age for the treatments feed zooplankton with (L-A) and without algae (L-NA). Error bars represent standard error of the mean ($n = 4$).

2.3. Statistical analysis

Growth data were analyzed using a two-way analysis of variance (ANOVA). The Student–Newman–Keuls multiple-range test (Steel and Torrie, 1980) was used to determine significant differences among treatments. Enzyme activity data were analyzed using two-way analysis of variance using a random effects model (Snedecor and Cochran, 1989). All significance levels were set at $P < 0.05$. Statistical analyses were performed using SAS System for Windows (v. 6.11, SAS Institute, NC).

3. Results

Growth, determined as the standard length of red drum larvae at a given age, was significantly higher in larvae reared in the presence of algae (L-A and M-A) than in

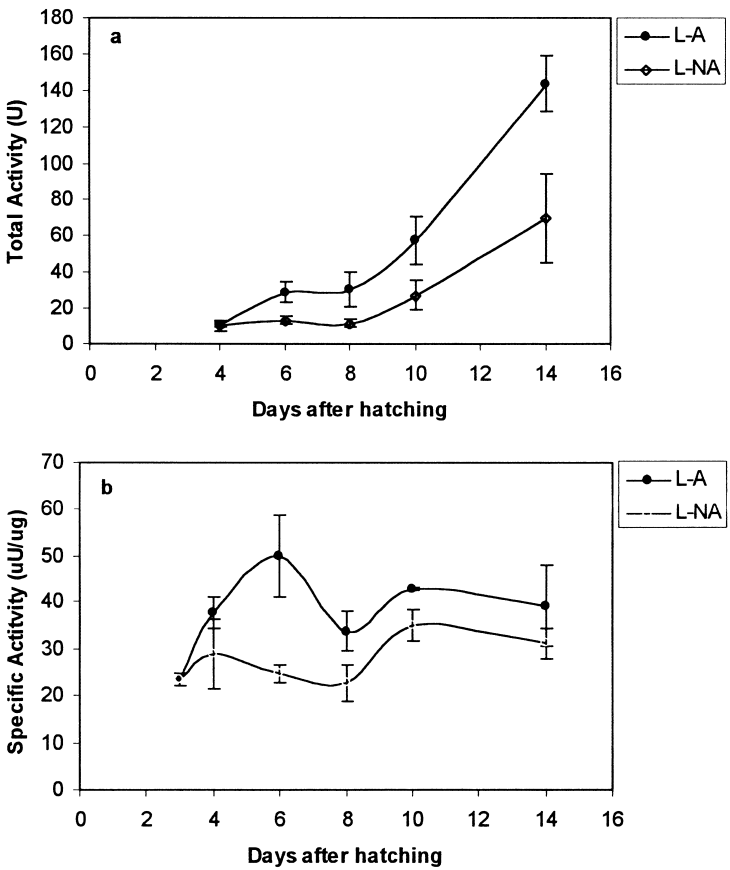

Fig. 3. Relationship between (a) total aminopeptidase activity and age, and (b) specific aminopeptidase activity and age for the treatments feed zooplankton with (L-A) and without algae (L-NA). Error bars represent standard error of the mean ($n = 4$).

larvae raised in the corresponding treatments without algae (L-NA and M-NA; Fig. 1). No significant differences in final standard length were observed between similar treatments in both feeding trials, therefore the data were pooled for statistical analysis. Two-way analysis of variance indicated that age and the presence or absence of algae had a significant effect on growth, although the interaction was not significant. At the end of the experiments, mean standard length of the larvae in the L-A treatment was 14% higher than larvae in the L-NA treatment. Similarly, larvae in the M-A treatment exhibited 22% higher standard length compared to the larvae in the M-NA treatment (Fig. 1). Survival was highly variable in all treatments, ranging from 4% to 14%. Mean final survival values were 30% higher for treatment L-A compared to L-NA (14.1% and 10.1%, respectively) and 42% higher in M-A than for M-NA (6.2% and 4.0%, respectively). However, survival was only significantly higher for the treatment M-A compared to M-NA.

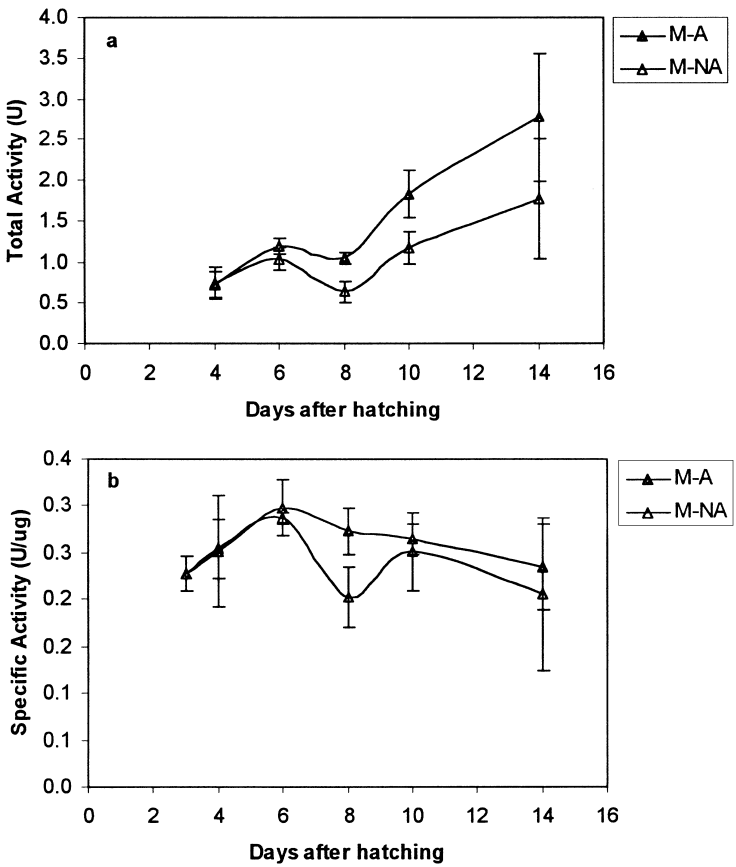

Fig. 4. Relationship between (a) total trypsin activity and age, and (b) specific trypsin activity and age for the treatments feed microparticulate diet with (M-A) and without algae (M-NA). Error bars represent standard error of the mean ($n = 4$).

In this study, we evaluated the effect of algal presence on digestive enzyme activity of red drum fed zooplankton or a microparticulate diet. Two-way analysis of variance indicated that age and algae had a significant effect on trypsin and aminopeptidase total activity (Table 2). The presence of algae resulted in a significant increase in total activity per gut (segmental activity as defined by Cahu and Zambonino-Infante, 1994; Peres et al., 1997) for trypsin and aminopeptidase. In terms of specific activity, age had a significant effect on trypsin but not on aminopeptidase. On the other hand, algae had an effect on the specific activity of aminopeptidase, but not on trypsin. Aminopeptidase specific activity was significantly higher only for the L-A treatment on day 6.

For clarity of interpretation, the results will be presented with respect to each diet type. Comparison of gut specific activities for larvae fed zooplankton indicated that the presence of algae resulted in an average (mean of the five sampling dates) increase in

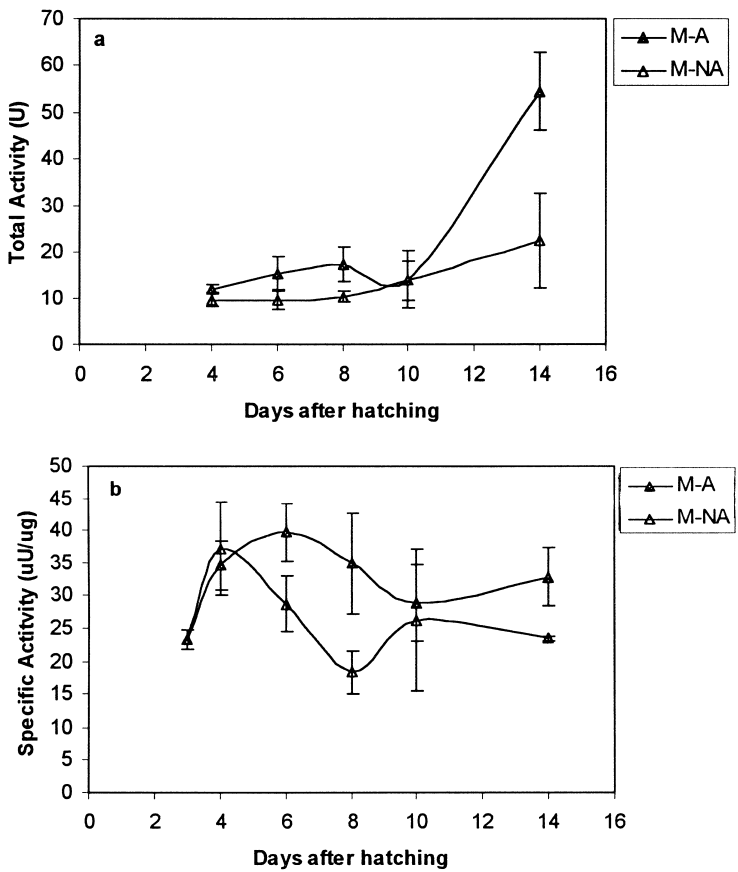

Fig. 5. Relationship between (a) total aminopeptidase activity and age, and (b) specific aminopeptidase activity and age for the treatments feed microparticulate diet with (M-A) and without algae (M-NA). Error bars represent standard error of the mean ($n = 4$).

specific activity of 27% and 46% for trypsin (Fig. 2) and aminopeptidase (Fig. 3), respectively. Similarly, larvae raised on the MP diet exhibited an average increase in the specific activities of larvae raised with algae of 11% and 34% for trypsin (Fig. 4) and aminopeptidase (Fig. 5), respectively.

Means $\pm$ standard deviation of ammonia-nitrogen and nitrite-nitrogen were 0.075 ± 0.040 and 0.045 ± 0.065 mg l^{-1}, respectively, for the live treatments and $0.337 \pm 0.097$and 0.180 ± 0.288 mg l^{-1}, respectively, for the tanks receiving microparticulate diet. The pH was 7.90 ± 0.10 for the live treatments and 7.80 ± 0.09 for the microparticulate treatments. These values are within the acceptable range for rearing red drum larvae (Holt et al., 1990). No effect of algae on water quality parameters was observed between diet types.

4. Discussion

The presence of algae in the rearing tanks improved growth of red drum larvae for both types of foods. To our knowledge, this is the first report of adequate growth exhibited by a marine fish species raised on practical microparticulate diet from first feeding without the use of zooplankton. Previously, red drum larvae have been gradually weaned onto a microparticulate diet at a very precocious stage (by day 8 post hatch) while maintaining similar growth and survival to larvae raised on zooplankton (Holt, 1993). Previous studies in our laboratory have shown that red drum fed solely on the microparticulate diet die by day 14 (Lazo et al., 2000). In this study, growth of red drum larvae raised on the microparticulate diet and algae was not significantly different from the zooplankton treatments (Fig. 1). The larvae fed the microparticulate diet in the absence of algae were significantly smaller than the other three treatments. The growth observed in the L-A treatment was comparable to the growth routinely obtained with red drum larvae fed zooplankton at our laboratory (Holt, 1993). Furthermore, although survival was relatively low in all treatments, the addition of algae improved survival for both diet types.

Our results, which were consistent for two separate feeding trials, suggest that red drum may be raised successfully to the juvenile stage by supplementing the microparticulate diet with algae in the rearing tanks. In ongoing research studies, red drum larvae fed the M-A treatment were raised successfully past metamorphosis to the juvenile stage (i.e., $>$ day 24), whereas the M-NA group died around day 14.

Differences in the specific activity of the enzymes measured in all treatments were only significant for aminopeptidase, and only the L-A treatment was significantly different. The digestive enzyme data presented here supports previous findings in our laboratory in which we found that the inability to successfully rear red drum larvae without live food could not be attributed to a poorly developed digestive system (i.e., enzymatic equipment) or the need for exogenous enzymes (Lazo et al., 2000). We showed that the specific activity of trypsin, lipase and amylase were not significantly affected by feeding the larvae with zooplankton or the same microparticulate diet used in this study. In the present study larvae in the M-A treatment had similar enzyme

activities for trypsin and aminopeptidase as larvae in the L-NA treatment, corroborating our previous conclusions that red drum larvae can produce similar enzyme levels whether fed a microparticulate diet or zooplankton.

Since the diffuse pancreas cannot be separated from the intestine in red drum larvae (Lazo, 1999), enzyme activities represent total enzyme production by the larvae (pancreas + intestine). Hence, even though red drum larvae may produce adequate amounts of digestive enzymes, it is possible that the microparticulate diet alone fails to stimulate secretion of the enzymes from the pancreas and that the presence of algae stimulates enzyme secretion and enhanced digestion.

Many compounds present in phytoplankton could potentially influence digestive enzyme activity in fish larvae. Polyamides, algae growth regulators, have been shown to stimulate cholecystokinin release in rats, which mediates the release of pancreatic enzymes (Fioramonti et al., 1994). Most formulated diets for marine fish larvae contain large amounts of fish meal, which is low in the polyamide spermine (Bardocz et al., 1993). The addition of spermine to the diets of European sea bass larvae has been shown to affect pancreatic enzyme secretion and to induce earlier intestinal maturation (Peres et al., 1997). In addition, amino acids may increase secretion of certain hormones, such as somatostatin and bombasin, which stimulate the secretion of pancreatic enzymes (Chey, 1993; Kolkovski et al., 1997). Cahu and Zambonino-Infante (1995a) observed increased trypsin secretion in European sea bass larvae fed a mixture of free amino acids in their diets.

Based on our growth results, we can assume that the assimilation of the microparticulate diet was significantly improved by the addition of algae. A significant increase in trypsin and aminopeptidase activity was observed, which would help in digestion of the diet if the enzymes were secreted. Increased trypsin and aminopeptidase activity have been suggested to improve growth or survival (Pedersen, 1993; Cahu and Zambonino-Infante, 1995a,b). An increase in specific activity of aminopeptidase, a brush border membrane enzyme, has been previously related to maturation of the intestinal membrane and enhanced survival in fish (Cahu and Zambonino-Infante, 1995b).

A direct cause and effect relationship cannot be elucidated from this study. Further and more detailed research is required to identify the mechanism through which algae stimulated the production and/or secretion of digestive enzymes, in addition to increasing growth and survival of red drum larvae. Nonetheless, in the hope of stimulating further research, several falsifiable conjectures that could further help explain the observed results are presented.

1. The presence of algae in the rearing tanks stimulated feeding behavior in red drum larvae. Many compounds, such as betaine, inosine 5-monophosphate and amino acids have been shown to stimulate feeding in fish (Metailler et al., 1983; Mearns, 1986; Knutsen, 1992; Kolkovski et al., 1997) and are natural constituents of phytoplankton or zooplankton (Dabrowski and Ruseick, 1983). Compounds such as betaine and particularly dimethylsulfoniopropionate (DMSP) can be released into solution ($>66\%$) as a result of zooplankton grazing (Christaki et al., 1996) or cell lysis due to algal death caused by bacteria or viruses (Mitsutani, 1997; Gillian et al., 1998). It is of particular interest to note that DMSP is a naturally occurring analog to betaine, which many marine phytoplankton utilize as an osmolyte (Kiene et al., 1998). Since betaine is

considered to be one of the most effective feeding stimulants, DMSP might act as an agonist to betaine in the chemical stimulation of feeding behavior in fish.

2. The algae provided a direct supply of nutrients. According to Moffat (1981), it is possible that algae may provide nutrients directly to the larvae. During early development, free amino acids play an important role in energy production and protein synthesis (Fyhn, 1993) and are contained in large amounts in algae (Hammer et al., 1981; Admiral et al., 1986). We have recently compared our M-A treatment using two different species of algae, *Isochrysis* vs. *Nannochloris*, that display different nutrient profiles (higher DHA content in *Isochrysis*; Volkman et al., 1989). We observed significantly higher growth and survival of larvae in the presence of *Isochrysis*. It is interesting to note that although larvae fed *Nannochloris* were smaller they still exhibited significantly higher growth and survival than red drum raised without algae. In addition to the nutritional effects of algae, the presence of algae may have affected larval gut and/or tank microflora (Gatesoupe, 1991; Skjermo and Vadstein, 1993; Oivind et al., 1994) or may have influenced the light regime of the tanks (Naas et al., 1992, 1996).

In summary, the presence of algae in tanks resulted in increased growth, survival and enzyme activity for both types of feeds. In addition, the growth of larvae fed the MP in the presence of algae was not significantly different from those fed zooplankton. Results demonstrated that red drum larvae may be raised on MP from first feeding without the use of rotifers or other zooplankton. The results obtained from the present study warrant a shift from the current paradigm, which proposes a lack of digestive enzymes during early development as an explanation for the inability to satisfactorily raise marine fish larvae on artificial diets. More recently, evidence is accumulating to support the idea of developing diets that properly induce secretion of digestive enzymes and present nutrients in a digestible form to successfully produce marine fish larvae under culture conditions

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References

Admiral, W., Peletier, H.R., Laane, P.M., 1986. Nitrogen metabolism of marine planktonic diatoms; excretion, assimilation and cellular pools of free amino acids in seven species with different cell size. J. Exp. Mar. Biol. Ecol. 98, 241–263.

Appel, W., 1974. Leucine aminopeptidase determination with L-leucineamide as substrate. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis. Academic Press, New York, pp. 954–958.

Arnold, C.R., 1988. Controlled year-round spawning of the red drum (*Sciaenops ocellatus*) in captivity. Contr. Mar. Sci. 30, 65–70.

Bardocz, S., Grant, G., Brown, D.S., Ralph, D., Pusztai, A.A., 1993. Polyamines in food-implications for growth and health. J. Nutr. Biochem. 4, 66–71.

Brinkmeyer, R., Holt, G.J., 1998. Highly unsaturated fatty acids in diets for red drum (*Sciaenops ocellatus*) larvae. Aquaculture 161, 53–268.

Cahu, C.L., Zambonino-Infante, J.L., 1994. Early weaning of sea bass (*Dicentrarchus labrax*) larvae with a compound diet: effect on digestive enzymes. Comp. Biochem. Physiol. 109, 213–222.

Cahu, C.L., Zambonino-Infante, J.L., 1995a. Effect of the molecular form of dietary nitrogen supply in sea bass larvae: response of pancreatic enzymes and intestinal peptidases. Fish Physiol. Biochem. 14, 209–214.

Cahu, C.L., Zambonino-Infante, J.L., 1995b. Maturation of the pancreatic and intestinal digestive functions in sea bass (*Dicentrarchus labrax*): effect of weaning with different protein sources. Fish Physiol. Biochem. 14, 431–437.

Cahu, C.L., Zambonino-Infante, J.L., 1998. Algal addition in sea bas (*Dicentrarchus labrax*) larvae rearing: effect on digestive enzymes. Aquaculture 161, 479–489.

Chey, W.Y., 1993. Hormonal control of pancreatic exocrine secretion. In: Go, V.L.W, Gardner, J.D., Brooks, F.P., Lebenthal, E., Di Magno, E.P., Sheele, G.A. (Eds.), The Pancreas; Biology, Pathology and Disease. Raven Press, New York, pp. 403–424.

Christaki, U., Belviso, S., Dolan, J.R., Corn, M., 1996. Assessment of the role of copepods and ciliates in the release to solution of particulate DMSP. Mar. Ecol. Prog. Ser. 141, 119–127.

Dabrowski, K., Ruseick, M., 1983. Content of total and free amino acids in zooplankton food of fish larvae. Aquaculture 30, 31–42.

Erlanger, B.F., Kokowsky, N., Cohen, W., 1961. The propagation and properties of two new chromatogenic substrates of trypsin. Arch. Biochem. Biophys. 95, 271–278.

Fioramonti, J., Fargeas, M.J., Bertrand, V., Pradayrol, L., Bueno, L., 1994. Induction of postprandial intestinal motility and release of cholecystokinin by polyamines in rats. Am. J. Physiol. 267, G960–G965.

Fyhn, H.J., 1993. Multiple function of free amino acids during embryogenesis in marine fishes. In: Walther, B.T., Jorgen-Fyhn, H. (Eds.), Physiological and Biochemical Aspects of Fish Development. University of Bergen, Norway, pp. 299–308.

Gatesoupe, F.J., 1991. Bacillus sp. spores as food additives for the rotifer Brachionus plicatilis: improvement of their bacterial environment and their dietary value for larval turbot, *Scophthalmus maximus* L. In: Kaushik, S.J., Luquet, P. (Eds.), Fish Nutrition in Practice. INRA Editions, Paris, pp. 561–568.

Gillian, M., Wilson, W.H., Bratbak, G., Liss, P., Mann, N.H., 1998. Elevated production of dimethylsulfide resulting from viral infection of cultures of *Phaeocystis pouchetti*. Limnol. Oceanogr. 43, 1389–1393.

Hammer, K.D., Brockman, U.H., Kattner, G., 1981. Release of dissolved free amino acids during a bloom of *Thalossiosira rotula*. Kiel. Meereforsch., Sonderh. 5, 101–109.

Holt, G.J., 1993. Feeding larval red drum on microparticulate diets in closed recirculating water system. J. World Aquacult. Soc. 42, 225–240.

Holt, G.J., Arnold, C.R., Riley, C.M., 1990. Intensive culture of larval and postlarval red drun. In: Chamberlain, G., Miget, R., Haby, M.G. (Eds.), Red Drum Aquaculture. pp. 53–56, Texas A&M Sea Grant College Program, US.

Kiene, R.P., Hoffman, W.L.P., Walker, J.E., 1998. Seawater microorganisms have a high affinity glycine uptake system which also recognizes dimethylsulfoniopropianate. Aquat. Microb. Ecol. 15, 39–51.

Knutsen, J.A., 1992. Feeding behavior of North Sea turbot *Scophthalmus maximus* and Dover sole *Solea solea* larvae elicited by chemical stimuli. Mar. Biol. 113, 543–548.

Kolkovski, S., Arieli, A., Tandler, A., 1997. Visual and chemical cues stimulate microdiet ingestion in seabream larvae. Aquacult. Int. 5, 527–536.

Kolkovski, S., Tandler, A., Kissil, G.W., Gertler, A., 1993. The effect of dietary exogenous digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream (*Sparus aurata*, Sparidae, Linnaeus) larvae. Fish Physiol. Biochem. 12, 203–209.

Lauff, M., Hoffer, R., 1984. Proteolitic enzymes in fish development and the importance of dietary enzymes. Aquaculture 37, 335–346.

Lazo, J.P., 1999. Development of the digestive system in red drum (*Sciaenops ocellatus*) larvae. PhD dissertation. The University of Texas at Austin, Austin, TX.

Lazo, J.P., Holt, G.J. , Arnold, C.R., 2000. Ontogeny of pancreatic enzymes in larval red drum (*Sciaenops ocellatus*) Aquacult. Nutr. 6 (in press).

Mearns, K.J., 1986. Sensitivity of brown trout (*Salmo trutta* L.) and Atlantic salmon (*Salmo salar* L.) fry to amino acids at the start of exogenous feeding. Aquaculture 55, 191–200.

Merchie, G., Lavens, P., Sorgeloos, P., 1997. Optimization of dietary vitamin C in fish and crustacean larvae: a review. Aquaculture 155, 165–181.

Metailler, R., Cadena-Roa, M., Person-Le Ruyet, J., 1983. Attractive chemical substances for the weaning of Dover sole (*Solea vulgaris*): qualitative and quantitative approach. J. World Maricult. Soc. 14, 679–684.

Mitsutani, A., 1997. Ecological and physiological studies on algae lytic bacteria in lakes and coastal waters. J. Natl. Fish.Univ. 45, 165–257.

Moffatt, N.M., 1981. Survival and growth of northern anchovy larvae on low zooplankton densities as affected by the presence of a *Chlorella* bloom. Rapp. P.-V. Reun. Cons. Int. Explor. Mer. 178, 475–480.

Naas, K., Huse, I., Iglesias, J., 1996. Illumination in first feeding tanks for marine fish larvae. Aquacult. Eng. 15, 291–300.

Naas, K., Naess, T., Harboe, T., 1992. Enhance first feeding of halibut larvae (*Hippoglossus hipoglossus*) in green water. Aquaculture 105, 143–156.

Oivind, O., Naas, K.E., Harboe, T., 1994. Shift in the intestinal microflora of Atlantic halibut (*Hippoglossus hipoglossus*) larvae during first feeding. Can. J. Fish. Aquat. Sci. 51, 1899–1903.

Pedersen, B.H., 1993. Protein digestion in herring (*Clupea harengus*) larvae: trypsinogen secretion and effects of a transitory food restriction on mortality, growth and digestive enzyme content. In: Walther, B.T., Jorgen-Fyhn, H. (Eds.), Physiological and Biochemical Aspects of Fish Development. University of Bergen, Norway, pp. 220–225.

Peres, A., Cahu, C.L., Zambonino-Infante, J.L., 1997. Dietary spermine supplementation induces intestinal maturation in sea bass (*Dicentrarchus labrax*) larvae. Fish Physiol. Biochem. 16, 479–485.

Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1994. Effect of short and long-term lipid enrichment on total lipids, lipid class and fatty acid composition in rotifers. Aquacult. Int. 2, 19–32.

Reitain, K., Rainuzzo, J.R., Oie, G., Olsen, Y., 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155, 207–221.

Skjermo, J., Vadstein, O., 1993. The effect of microalgae on skin and gut bacterial flora of halibut larvae. In: Dahle, L.A., Jorgensen, L., Tvinnereim, K. (Eds.), Proceedings of the International Conference on Fish Farming Technology, Trondeheim, Norway.

Snedecor, G.W., Cochran, W.G., 1989. Statistical Methods. 8th edn. Iowa State University Press, Ames, IA, USA.

Spotte, S., 1979. Fish and invertebrate culture: water management in closed systems. 2nd edn. Wiley, NY, USA.

Steel, R.G., Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biometrics Approach. McGraw-Hill, New York, NY, USA.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 128, 219–240.