嘉南藥理科技大學專題研究計畫成果報告

餌料添加誘餌劑對石斑幼苗之存活評估

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計畫主持人：王瑞顯

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Evaluation of survival on grouper (*Epinephelus* sp.) larvae fed on attractants

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中文摘要

添加數種單一誘餌劑(glutamic acid, taurine, sucrose, betaine)至基本餌料，然後餵食石斑幼苗28天，以評估其生長與存活率。結果以活豐年蝦餌料之餵食得到石斑幼苗最佳之最終生長重量(52.6 mg)與存活率(54.3%)最佳，在合成餌料中以0.5%之sucrose添加組，其最終百分比存活率(32.6%)與基本餌料組(35.3%)相近，且顯著高於其它誘餌劑添加組($p < 0.05$)，而最終生長重量(30.9 mg)亦顯著高於其它各誘餌合成餌料組($p < 0.05$)。

關鍵詞: 石斑幼苗、誘餌劑、存活率。

ABSTRACT

Individual attractants (glutamic acid, taurine, sucrose, betaine) were added as a supplement to a basal diet and fed to the grouper larvae (*Epinephelus* sp.) for the evaluation of its growth and survival. Each group of striped bass larvae was evaluated during a 28-d feeding trial. The live *Artemia* nauplii diet fed to the larvae had the best performance for growth and survival in the present feeding trials. The combination of sucrose, glutamic acid and glycine with 0.125% each of supplemented diet was found to be significantly lower ($p < 0.05$) in percentage cannibalism (3.3%) and higher final percentage survival (28.0%) than all other formulated groups.

Keywords: Grouper larvae, *Epinephelus* sp., attractants, survival.

INTRODUCTION

The grouper (*Epinephelus* sp.) is an important recreational and commercial fish species in Taiwan. Due to its high economic value, aquatic farmers have been made to culture this valuable fish species through private and governmental hatcheries in order to enhance populations.

Live zooplankton, especially live brine shrimp (*Artemia*) nauplii, usually provide the best performing diet for rearing the larval stages of fish and crustaceans, since it is easily obtained or cultured, has an appropriate size and is nutritionally adequate (Simpson et al. 1983; Leger et al. 1986). However, various reports have stated that using *Artemia* nauplii resulted in poor larval rearing, e.g., chlorinated hydrocarbon contamination (Olney, 1980) or lack of essential fatty acids (Schauer et al. 1980;

Leger et al. 1985). Artificially formulated diets were thus subsequently essential and important for incorporation of nutrient requirements for aquatic animals.

Macronutrients are major contributors of nutritional values for aquatic fish. The attractiveness of the food in terms of palatability, odor and taste significantly influence the growth and survival of small fish when they first begin to feed (Lemm and Hendrix, 1981). Usually, the attractants that are common to aquatic animals are the amino acids, nucleic acid related compounds, quaternary ammonium bases and carbohydrates related compounds (Lindstedt, 1971; Harada, 1990). Various studies using dietary supplements of specific attractants have shown stimulatory effects to fish appetites, thus increasing feeding efficiency, digestion, absorption and growth in various aquatic fish (Ardon and Mackie, 1978; Tandler et al., 1982; Murai et al., 1985; Takii et al., 1986).

Effective utilization of formulated or nonliving diets for the growth of this larva has not yet been successfully achieved. No studies so far have been conducted for using various attractants to rear the valuable striped bass. Thus, various attractants (glutamic acid, taurine, sucrose, betaine) were added to grouper basal diet for the purpose of elevating growth and survival of this fish species through feeding trials.

MATERIALS AND METHODS

iltered seawater (0.45 μm) of 30 ‰ salinity was obtained from a local aquaculture farm. One gram of each dry *Artemia* cysts, RAC *Artemia* (Reference *Artemia* cysts) was separately hatched in a separatory glass funnel containing 2 L of filtered seawater (30‰ salinity) under continuous strong aeration at 25 ± 2°C for 24-h with light. After 24-h hatching, *Artemia* nauplii were harvested under a 106 μm sieve and transferred to 10L glass tanks containing seawater. Fifty grouper at two-wk-old (obtained from a local aquaculture farm) were randomly collected and transferred to each of the 10L glass tanks under moderate aeration (20 ± 2°C). All the treatments were conducted replicated twice.

The components of basal diet used in the present feeding trial were the same as the report of Wang et al. (1995) shown in Table 1. For the processing of the attractant supplemented diets, individual or combinations of attractants at 0.5% of the total dry weight was spread on the top of the pellets, and then the pellets were mixed and passed through the grinder again. The different pellet diets were then dried at 40°C with hot blowing air for 4 hr to obtain a constant moisture content. The dry pellets were then crumbled and passed through a 500 μm sieve then collected at a 250 μm to obtain 250-500 μ m particles. These prepared diets were then stored in the -4°C freezer, ready for feeding the fish larvae.

A ration in dry weight of diets of 100% of fish wet weight was used for each diet. After 28 days of feeding, fish larvae were collected, blotted dry and measured for their weight and length. Survival and

growth conditions of the fish larvae were analyzed by one way analysis of variance and Duncan's multiple range test for the significant differences among the different diets.

RESULTS AND DISCUSSION

Final percent survivals on each treatment are shown in Table 2. Less than 35.3% final survival was found in all treatments with formulated diets. The prepared diet containing a sucrose (0.5% each) had the highest final percent survival (32.6%) among the attractant treatments. Among these treatments, the 0.5% betaine additative diet showed the significantly lowest ($p < 0.05$) final survival (17.2%) of any diet. The highest percent cannibalism was also found in the betaine supplemented diet (20.5%) and lowest in the taurine supplemented diet (13.3%).

In most aquaculture hatcheries, an average survival of 30-40% is normal for aquatic larvae. In the present experiment, larvae fed with *Artemia* nauplii dominated those fed formulated diets for growth and survival. Some unknown growth factors existing in nauplii may contribute to their superior performance in rearing striped bass larvae (Baragi and Lovell, 1986). A similar recent study was conducted in this laboratory based on the same ingredients supplemented with a protease enzyme, which resulted in percent survivals ranging from 30.6%-37.3% (Wang et al., 1995). Most of the percent survivals for formulated diets in the present feeding trial were in the lower range of less than 35.3%. However, the average final body weights (wet wt.) from formulated diet treatments (14.3-30.9 mg) in this trial were about three times greater than those found in the study of Wang et al. (1995), ranging from 9.7-11.2 mg. This difference may be due to the severe cannibalism in the present feeding trial. According to various reports, cannibalism was frequently found in intensive culture or closed systems of striped bass larvae (Braid and Shell, 1981; Braid, 1974; Paller and Lewis, 1987). Cannibalism occurring in this study may cause fewer number of existing fish to have a greater chance to capture food, thus increasing their weight gain.

The percent survivals of grouper larvae in this feeding trial were mostly higher than the study by Webster and Lovell (1990). During the 19-d posthatch feeding experiment in that study, survival for striped bass was greatest for fish fed with live *Artemia* (28.5%), followed by fish fed a dry diet (18.0%), freeze-dried *Artemia* (9.8%) and shock-frozen *Artemia* (9.3%). Thus, the formulated basal diet used in the present feeding trial can provide minimally satisfactory nutrition for the survival and growth of grouper larvae.

The individual attractant treatments did not give satisfactory survival or growth performance compared to the basal diet (Tables 2). Various studies revealed that mixtures of attractive substances have advantages for the rearing of fish (Mackie et al., 1980; Cadena-Roa et al., 1982; Harada, 1990). Also, Métailler et al (1983) pointed out that a mixture of attractants

(4.36% betaine HCl, 1.38% L-glycine, 0.77% alanine, 0.25% arginine and 0.52% L-glutamic acid) caused a higher survival rate than the other mixtures for the rearing of Dover sole. According to Hashimoto et al. (1968), the stimulating activity of short-necked clam extracts (attractants) is attributable mainly to the synergistic or additive interaction between amino acids. In this study that the glycine added to the sucrose and glutamic acid mixture contributes to the higher final survival (28.0%) compared to that of the individual attractant, sucrose (13.3%), glutamic acid (16.0%) or the combination of sucrose and glutamic acid (12.0%).

It is also apparent that different species of fish may detect and respond to somewhat different chemical clues emanating from a specific food type (Carr, 1976). Hara (1973) reported that glycine had a significantly higher attractant value for rainbow trout than L-glutamic acid and L-lysine. However, catfish had olfactory receptors more sensitive to L-glutamic acid than to glycine (Suzuki and Tucker, 1971). A 0.9% betaine additive was significantly better for survival and growth in lake whitefish larvae (Zitzow and Millard, 1988). Nevertheless, the 0.5% betaine supplemented diet used in this study showed the lowest survival (17.2%). The results from this experiment may demonstrate that certain attractants inactive or repellent to the larvae in these feeding trials.

The concentrations of the attractants used are applied with only one concentration in each treatment under this feeding trial. According to Tandler et al. (1982), basal diet supplemented with 10 g/kg (1%) or 20 g/Kg (2%) of synthetic muscle extract is strongly associated with higher appetite levels than the basal diet or 5 g/Kg (0.5%) extract supplemented diets. No investigation of various concentration levels of attractants were conducted in this present study. For future study, the appetite dependency on alternative attractant concentrations should also be evaluated and compared.

Table 1. Percent of ingredients and proximate composition of the formulated diets in this feeding experiment.

Ingredients	%(dry basis)
Krill meal (58% Protein)	56
Whole egg solids	25
Plankton fines	5
Bovine liver extract	2
Yeast autolysate	2
Lecithin	2
Menhaden oil	3
Betaine	2
Sea salt	1.5
Gum binder	1
Trace mineral mixture	0.5
Vitamin mixture	1
Ascorbyl-2-polyphosphate	0.01
Attractants added	%
Betaine	0.5
Taurine	0.5
Glutamic acid	0.5
Sucrose	0.5

Table 2. Average final percentage survival and cannibalism of grouper larvae in this 28-day experiment.

Treatments	Final % survival	Final body weight (mg)	% Cannibalism†
Artemia	54.3[a]*	52.6[a]	14.6[a]
Basal diet	35.3[b]	27.5[b]	13.8[a]
Betaine	17.2[c]	14.3[c]	20.5[b]
Taurine	21.8[c]	20.1[d]	13.3[a]
Glutamic acid	19.1[c]	22.4[d]	15.6[a]
Sucrose	32.6[b]	30.9[b]	14.9[a]

*Differe nt letters denote pairs of groups significantly different at the 0.05 level for Duncan's multiple range test.

†% Cannibalism = (initial # of fish-total # of mortality fish recorded)/initial # of fish