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Effect of Different Dietary Protein and Lipid Levels on Growth Performance and Body Composition of Juvenile Southern Flounder, *Paralichthys lethostigma*, Reared in a Recirculating Aquaculture System

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Abstract

The effects of six formulated diets containing different protein and lipid levels on growth performance and body composition of juvenile southern flounder were evaluated. Test diets were prepared with a combination of three crude protein (CP) levels (45, 50 and 55%) and two crude lipid (CL) levels (10 and 15%). Diets (CP/CL) were as follows: 45/10, 45/15, 50/10, 50/15, 55/10, 55/15 and a commercial diet (50/15). Southern flounder (1.10 g) were fed the respective diets for 42 d in triplicate recirculating tanks (20 fish/tank). Percent body weight gain (BWG) for fish fed diet 45/10 (413%) and the commercial diet (426%) were significantly ($P < 0.05$) lower than fish fed other diets (823–837%). Increasing protein level from 45 to 50% produced a significant increase in BWG for the 10% lipid diet (823%) but further increasing protein did not produce a significant effect on BWG irrespective of dietary lipid levels. Specific growth rate (SGR), feed intake, feed conversion efficiency (FCE), protein efficiency ratio (PER), and total lipid content in the whole body were significantly affected by different dietary protein and lipid levels. Results indicated that a combination of 50% protein and 10% lipid was optimal for the growth performance of southern flounder juveniles.

The southern flounder, *Paralichthys lethostigma* (*P. lethostigma*), is a flatfish of the family Bothidae. It can be found in coastal waters from Albemarle Sound, North Carolina, through South Atlantic states to Corpus Christi Pass, Texas, with the exception of South Florida (Wenner et al. 1990). This species can grow well in seawater or in freshwater. Their euryhaline character and tolerance of a wide range of temperatures make southern flounder an ideal candidate for aquaculture. The development of intensive culture methods for southern flounder is of great interest because of its status as a highly desirable food and recreational species and its potential for commercial culture. Its landings have declined, leading to interest in culturing native flatfishes for stock enhancement or food fish production. Various institutions in North Carolina are devoting substantial resources to address the major problems associated with the mariculture of this species, particularly in recirculating aquaculture system (Daniels and Watanabe 2003). The methodology of spawning and larval rearing is well documented (Berlinsky et al. 1996; Watanabe et al. 2001; Daniels and Watanabe 2003; Watanabe et al. 2006). One of the factors limiting commercial aquaculture production of southern flounder is the limited researches regarding the nutritional requirements (Alam and Watanabe 2005; Gao et al. 2005; Gonzalez et al. 2005).

Feed is one of the highest costs in the operation of an aquaculture enterprise, and in many cases reaches 50% of total expenses. Protein influences the economics of a farming industry by determining the feed cost (NRC 1993). Lipid is the source of energy for fish, and if lipid level is not sufficient in the diet as an

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energy source, protein is used as compensatory energy source. Therefore, dietary protein and lipid levels must be in balance for maximum growth of fish (Kim et al. 2004). It is important to provide an adequate level and ratio of protein, lipid, and carbohydrate in diets in order to reduce catabolism of protein for energy. The protein-sparing effects by lipid or carbohydrate levels in diets have been reported in some fish species (Cho and Kaushik 1990; De Silver et al. 1991; Lee et al. 2002).

Although the dietary protein requirement for southern flounder had been studied (Gao et al. 2005), no information is available on utilization of lipid as an energy source at different dietary protein levels. The objective of this study was to determine the effects of various levels of lipid and protein in diets on growth and body composition of juvenile southern flounder.

Materials and Methods

Experimental Animal

Adult broodstock southern flounder held in photothermally controlled tanks at the University of North Carolina Wilmington Center for Marine Science (UNCW-CMS) Aquaculture Facility, Wrightsville Beach, North Carolina were induced to spawn using luteinizing hormone releasing hormone analog (LHRHa) (Berlinsky et al. 1996; Watanabe et al. 2001; Watanabe et al. 2006). The fertilized eggs were hatched and reared through 70-d post hatching at the North Carolina State University fish hatchery (Raleigh, NC) as described by Daniels and Watanabe (2003) and then transferred to UNCW-CMS for experimental studies. Prior to the feeding experiment, fish were conditioned for 2 wk to a commercial pellet diet (Skretting, Canada, 1.0 mm pellet, crude protein [CP] 50%, and lipid 15%).

Experimental Diets

To study the effects of dietary protein and lipid on southern flounder, a 3×2 factorial experiment was designed. Six experimental diets were formulated to contain three protein (45, 50, and 55%) and two lipid (10 and 15%) levels. CP and crude lipid (CL) in the diets were as follows: 45/10, 45/15, 50/10, 50/15, 55/10, and 55/15 (Table 1). In addition, a domestic generic commercial diet (50% CP and 15% CL) was compared with the formulated practical diets. The protein sources of the formulated diets were menhaden meal, squid meal, and krill meal. Menhaden oil and soybean lecithin were used as lipid sources. Menhaden meal was increased to increase the protein level in diet while menhaden oil was increased to provide a higher lipid level in the diets. Wheat starch was used as a carbohydrate source to increase energy level. All other ingredients were formulated according to the recent studies on nutrient requirements of other marine flatfish (Alam et al. 2002, 2003). All ingredients were purchased locally except vitamin and mineral premix (Kohkin Chemical Co., Ltd, Kagoshima, Japan, Kagoshima University) and vitamin and mineral for marine fish. Protein sources were sieved through a 500-µm mesh sieve. Energy levels of the diets were calculated based on 16.7, 37.7, and 16.7 kJ/g for protein, lipid, and nitrogen-free extract, respectively (Garling and Wilson 1976). Diets were prepared as described previously (Alam et al. 2007), with some modifications. In brief, all dry ingredients were mixed with a feed mixer (Kitchen Aid Inc., St. Joseph, MI, USA), and then to this, previously mixed menhaden oil and lecithin were added. Approximately 35–40% of distilled water was added to the ingredient mixture to facilitate pelleting by a meat chopper (model MIN0012, Jacobi-Lewis Co., Wilmington, NC, USA). After pelleting, diets were dried at 70 C in a constant temperature oven (DKM 600, Yamato Scientific Co., Ltd., Japan) and then stored at −20 C. The proximate composition of the diets was analyzed (Table 2).

Experimental Conditions

To begin the experiment, southern flounder (approximately 1.10 g initial weight) were placed in triplicate recirculating tanks (75 L, 20 fish/tank). The recirculating aquaculture system included a Kaldness moving beds-based (Anox Kaldness Inc., Providence, RI, USA) biofilter, a

TABLE 1. *Composition of diets.*

Protein/lipid	Test diets g/100 g 45/10	45/15	50/10	50/15	55/10	55/15	Commercial 50/15
Menhaden meal[a]	39	39	47	47	54.5	54.5	
Squid meal[a]	20	20	20	20	20	20	
Krill meal[a]	5	5	5	5	5	5	
Menhaden oil[a]	2	7.5	1.5	6.5	1	5	
Soybean lecithin[b]	1.5	1.5	1.5	1.5	1.5	1.5	
Vitamin mix[c]	4	4	4	4	4	4	
Mineral mix[c]	4	4	4	4	4	4	
Wheat-starch[d]	18.5	13	11	6	4	0	
Gluten[d]	5	5	5	5	5	5	
Attractants[e]	1	1	1	1	1	1	
Total	100	100	100	100	100	100	
Gross energy (GE) (calculated, kJ/g diet)	14.0	15.5	14.1	15.1	14.0	14.5	
P/E ratio (mg protein/kJ GE)	32.1	29.0	35.4	33.1	39.2	37.9	

[a]International Proteins Corporation, St. Paul, MN.
[b]ADM Company, Decatur, IL.
[c]Kohkin Chemical Co., Ltd, Kagoshima, Japan (Alam et al. 2008).
[d]Sigma-Aldrich, St. Louis, MO.
[e]Attractants; alanine, betaine, glycine, and taurine (0.25% of each).

TABLE 2. *Proximate compositions of diets. Values are average of triplicate analysis ($N = 3$). (Commercial diet contained 50% crude protein, 15% lipid, 8% ash, 10% moisture).*

	45/10	45/15	50/10	50/15	55/10	55/15
Protein	45.5	45.9	50.2	51.0	54.7	54.9
Lipid	9.9	15.4	10.2	15.2	9.9	14.5
Moisture	10.1	11.2	10.3	12.4	10.5	12.3
Ash	10.2	11.0	11.4	12.5	12.9	13.6

bead filter (Aquaculture Systems Technologies, LLC, New Orleans, LA) to remove solids, a protein skimmers for removal of small particulate and dissolved materials and an ultraviolet (UV) sterilizer for disinfection. Fish were fed twice/d 0900 and 1600 h as much as they could consume during a 20-min period, and the amount of diet consumed was recorded daily. Each tank was covered with a lid to prevent fish from jumping out. A 10:14 h light-dark photoperiod was maintained. Water quality was checked twice weekly. Water temperature ranged from 19.1 to 20.5 C during the feeding period and dissolved oxygen (6.5–7.8 mg/L) was maintained near saturation with continuous aeration. Ranges of other parameters were pH 7.7 to 8.0 and salinity 33.5 to 34.4 ppt. Fish were weighed every 2 wks. Growth study was conducted for a period of 6 wks. A pooled sample of 10 fish at the beginning and 10 fish per tank at the end of the feeding trial were sacrificed and used to determine the whole body proximate composition.

Proximate Composition and Statistical Analyses

CP (nitrogen combustion) and crude fat content (ether extraction) and moisture and ash content of the diets and fish whole body were determined using AOAC (1990) method at New Jersey Feed Laboratory Inc., Trenton, New Jersey. All data were subjected to statistical verification using one way and two-way ANOVA (JMP, version 6.0, SAS Institute Inc., Cary, NC, USA). Significant differences between means were evaluated by Tukey Kramer test (Kramer 1956). Probabilities of $P < 0.05$ were considered significant.

Results

Effects on Growth Performance

After 14 d no significant ($P > 0.05$) differences in mean final body weight (FBW)

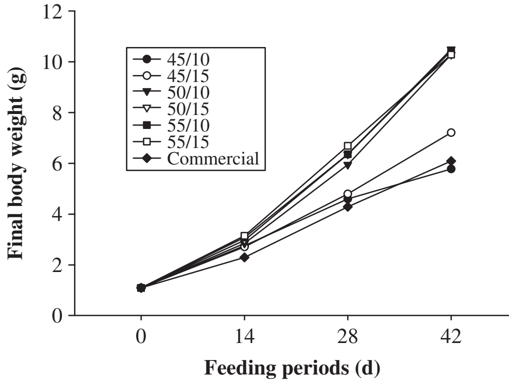

FIGURE 1. *Effect of different protein and lipid levels on final body weight (g) after 14, 28, and 42 d feeding trial. Values are means of triplicate tanks.*

among treatments were observed (Fig. 1). After 28 d, FBW among the juveniles fed 45/10 and 45/15 and the commercial diet were significantly ($P < 0.05$) lower than those fed 50/10, 50/15, 55/10, and 55/15 and similar trends were observed after 42 d (Fig. 1). The percent body weight gain (BWG), specific growth rate (SGR), feed intake (FI), feed conversion efficiency (FCE), protein efficiency ratio (PER), and percent survival (SR) are presented in Table 3. The lowest BWG (413%) was found for juveniles fed the 45/10 diet. At this protein level, increasing lipid levels from 10 to 15% significantly increased BWG (545%). Increasing protein levels from 45 to 50% increased BWG at both lipid levels to 823 and 830% respectively. Further increasing protein level from 50 to 55% did not significantly increase BWG irrespective of lipid levels. After 42 d, a significant ($P < 0.01$) interactive effect between dietary protein and lipid levels on BWG was observed (Table 3). SGR showed similar trends as observed for BWG data with lowest SGR at 45/10 and 45/15, but with no significant difference among the diets 50/10, 50/15, 55/10, and 55/15. FI was not significantly different among fish fed diets 45/10 and 45/15; however, FI was significantly higher for the juveniles fed 50/15, 55/10, and 55/15 (7.49–7.83). FCE showed the same trends as BWG. FCE was significantly lower ($P < 0.05$) on the 45% protein diets than on 50 or 55% protein. At 45% protein, FCE was significantly ($P < 0.05$) higher at 45/15 (0.97) than at 45/10 (0.76). However, there were no significant differences among 50/10, 50/15, 55/10, and 55/15 (1.17–1.29). The lowest FCE (0.76) was observed for fish fed 45/10 with no significant difference from the commercial diet (0.87).

TABLE 3. *Body weight gains (BWG), feed intake (FI), feed conversion efficiency (FCE), percent survival (SR), specific growth rate (SGR), and protein efficiency ratio (PER) of juvenile southern flounder fed diets with different levels of crude protein and crude lipids (CP/CL) for 42 d. Values are mean ± SEM for triplicate tanks. Means with different letters in the same column differ significantly (*$P < 0.05$*).*

Diets (CP/CL)	BWG (%)	FI g/fish/42 d	FCE	SR (%)	SGR %/d	PER
45/10	413 ± 12.7^{c}	$6.16 \pm 0.18b^{c}$	0.76 ± 0.04^{c}	94 ± 0.33^{a}	3.88 ± 0.06^{c}	1.51 ± 0.08^{c}
45/15	545 ± 21.6^{b}	6.26 ± 0.22^{bc}	0.97 ± 0.03^{b}	96 ± 1.00^{a}	4.41 ± 0.08^{bc}	1.94 ± 0.05^{b}
50/10	823 ± 20.8^{a}	7.13 ± 0.30^{ab}	1.29 ± 0.03^{a}	97 ± 0.05^{a}	5.26 ± 0.06^{a}	2.31 ± 0.05^{a}
50/15	830 ± 32.9^{a}	7.49 ± 0.23^{a}	1.25 ± 0.06^{a}	94 ± 1.15^{a}	4.96 ± 0.27^{ab}	2.25 ± 0.06^{ab}
55/10	837 ± 34.8^{a}	7.56 ± 0.16^{a}	1.23 ± 0.02^{a}	98 ± 1.15^{a}	5.33 ± 0.09^{a}	2.01 ± 0.09^{ab}
55/15	834 ± 12.9^{a}	7.83 ± 0.20^{a}	1.17 ± 0.05^{a}	98 ± 1.33^{a}	5.31 ± 0.03^{a}	1.91 ± 0.02^{b}
Com	426 ± 36.3^{c}	5.63 ± 0.25^{c}	0.87 ± 0.03^{c}	95 ± 1.52^{a}	3.91 ± 0.17^{c}	1.53 ± 0.09^{c}
Two-way ANOVA (P-value)						
Protein (A) 0.0001	0.0001	0.0001	0.0265	0.001	0.0003	
Lipid (B)	0.0851	0.1739	0.3702	0.8128	0.5811	0.2898
A × B	0.0297	0.8417	0.0037	0.0835	0.0203	0.0021

BWG (%) = ([final wet weight − initial wet weight]/initial wet weight) × 100.
SGR = (ln [mean final weight] − ln [mean initial weight]/42 d) × 100.
FCE = Total feed intake (g)/wet weight gain (g).
PER = weight gain (g)/total protein intake in dry basis (g).

PER also showed similar trends to BWG. PER was generally lower on the 45% protein diets than on 50 or 55% protein. At 45% protein, PER was significantly ($P < 0.05$) higher at 45/15 (1.94) than at 45/10 (1.51), while there were no significant differences among 50/10, 50/15, and 55/10 (2.01–2.31). PER in the 55/15 (1.91) diet was not significantly different from 45/15 (1.94). PERs were significantly lower for the 45/10 (1.51) and commercial diet (1.53) than for the other diets. After the feeding trial, a highly significant ($P < 0.01$) interactive effect between dietary protein and lipid levels on feed conversion ratio (FCE) and PER was observed but not for FI (Table 3). Survival (94 to 98%) was not significantly different among treatments.

Effects on Body Proximate Compositions

Whole body moisture (77.8–78.1%) and protein content (15.7–16.6) were not significantly different among treatments (Table 4). Body lipid content was significantly ($P < 0.05$) affected by dietary protein and lipid level. Lipid content of fish fed the high-lipid diets (2.31–2.64%) was significantly higher than for those fed the low-lipid diets (1.51–1.98%) regardless of dietary protein level (Table 4). Ash content (3.49–4.26%) was not significantly different among treatments. No interaction ($P > 0.05$) between protein and lipid level on whole body moisture, protein, and ash content of fish were observed, but a significant ($P < 0.05$) interaction was observed for whole body lipid content.

Discussion

In the present study, the poor growth performance of southern flounder juveniles fed the 45/10 diet could be as a result of a deficiency of protein. Insufficient dietary protein reduces protein assimilation and growth in fish (NRC 1993). The BWG for fish fed 45/15 diet was significantly higher than those fed 45/10, probably because of the increase in energy level in diet from lipid. In several fish species fed low protein diets, using or adding high energy containing lipid as a major energy source improved the growth (Vergara et al. 1996). The present finding is consistent with the idea that increasing dietary energy by lipid supplementation has a protein-sparing effect, allowing a more efficient use of protein, while reducing N excretion to the environment (Bureau et al. 2002). Protein sparing has been demonstrated in hybrid-striped bass, *Morone chrysops* × *Morone saxatilis* (Nematipour et al. 1992); red sea bream, *Pagrus major* (Takeuchi et al. 1991); gilthead sea bream, *Sparus aurata* L. (Vergara et al. 1996); turbot, *Scophthalmus maximus* (Bromley 1980); and Atlantic halibut, *Hippoglossus hippoglossus* (Aksnes et al. 1996; Helland and Grisdale-Helland 1998).

At a dietary lipid level of 10%, increasing protein level from 45 to 50% significantly increased BWG. However, further increasing protein or lipid levels did not affect BWG. The dietary protein requirement of juvenile southern flounder is 50.3% using menhaden meal as protein source and a dietary lipid level of 14% when reared in a recirculating system (Gonzalez et al. 2005). Gao et al. (2005) suggested that the optimum protein level in diets for juvenile southern flounder was 51.2% using fish meal and casein–gelatin as protein sources when reared in a net cage system supplied with flow through system. In the present study, increasing lipid levels from 10 to 15% in a 50% protein diet had no significant affect on BWG reared in a recirculating system. When fish are fed a diet containing excess lipid, growth may be reduced because of an imbalance of digestible energy/CP ratio and excessive fat deposition in the visceral cavity and tissues (Lovell 1989). However, increasing lipid level from 10 to 15%, did not decrease BWG in the present study. Danielssen and Hjertnes (1993) found no adverse effects of dietary fat levels up to 22% on growth of juvenile turbot. Increasing the lipid level from 8 to 20% in Atlantic halibut diets (Berge and Storebakken 1991), from 11 to 21% in Senegalese sole, *Solea senegalensis*, Kaup diets, (Dias et al. 2004), from 3.3 to 16.4% in Japanese flounder, *Paralichthys olivaceus* diets did not produce significant differences in weight gain. However, Kim et al. (2004) reported that in juvenile

TABLE 4. *Effects of dietary levels of protein and lipids on body proximate composition (% wet basis) of southern flounder. Values are mean ± SEM (N = 3). Means with different letters in the same column differ significantly (P < 0.05).*

Diets (CP/CL)	Moisture	Protein	Lipid	Ash
45/10	78.4 ± 0.17	16.0 ± 0.29	1.68 ± 0.16^{bc}	4.26 ± 0.13
45/15	77.8 ± 0.21	16.1 ± 0.51	2.54 ± 0.19^{ab}	3.84 ± 0.17
50/10	78.8 ± 0.27	16.5 ± 0.23	1.51 ± 0.11^{c}	3.64 ± 0.28
50/15	77.8 ± 0.26	16.6 ± 0.38	2.31 ± 0.17^{ab}	3.93 ± 0.29
55/10	78.0 ± 0.39	16.4 ± 0.50	1.98 ± 0.18^{abc}	3.79 ± 0.33
55/15	78.1 ± 0.08	15.7 ± 0.21	2.64 ± 0.11^{a}	3.49 ± 0.11
Com (50/15)	78.1 ± 0.05	16.3 ± 0.15	2.32 ± 0.10^{ab}	3.75 ± 0.66
Two-way ANOVA (*P*-value)				
Protein (A)	0.0394	0.4221	0.0362	0.9879
Lipid (B)	0.0026	0.2469	0.0007	0.1521
A × B	0.2907	0.5070	0.0062	0.5933

Japanese flounder, increasing lipid level from 6.3 to 12.9% in diets with more than 50% protein caused a significant reduction of growth. We assume that a diet containing 50% protein and 10% lipid was an appropriate combination for the growth of southern flounder under the present conditions. These levels were close to the reported optimum dietary protein and lipid levels for Japanese flounder (Kikuchi and Takeuchi 2002; Kim et al. 2002; Alam et al. 2003). This study also confirms that given the optimum dietary protein the ability to utilize lipid by southern flounder is low, similar to what was reported in Japanese flounder (Lee et al. 2000a; Alam et al. 2003).

BWG for the juveniles fed the commercial diet which contained 50% protein and 15% lipid was significantly lower than the formulated 50/15 diet. This could be because of the differences between these diets in sources of protein and lipid and other nutrients such as vitamins, minerals, carbohydrates which are not known for commercial diet.

In this study, FI for the juveniles was not significantly different among the formulated diets (6.16–7.83 g/fish/42 d) but was significantly lower for the commercial diet (5.63). This is probably because formulated diets contained similar energy levels (14.0 to 15.0 kJ/g diet) and fish eat to satisfy their energy requirement (Lee and Putnam 1973). The commercial diets often included plant meals that give a different palatability and texture to the pellets, as well as low-diet digestibility, and this may have caused the observed lower FI and BWG in the fish fed commercial diet. The highest FCE (1.29) observed for the 50/10 diet was similar to the reported value for the other flatfish fed fish meal-based diets (Lee et al. 2000b).

PER increased with increasing lipid level from 10 to 15% in the 45% protein diet. However, increasing protein from 50 to 55% caused PER to decrease at both lipid levels, which is consistent with other studies showing a decrease in PER with increasing dietary protein beyond the optimum level (McGoogan and Gatlin 1999; Thoman et al. 1999). A proper P/E ratio is important in formulating commercial feed. In the present study, dietary P/E ratio ranged from 29.0 to 39.2 mg protein/kJ diet among treatments. The optimum P/E ratio for the Japanese flounder was 27.5 mg protein/kJ diet as reported by Kim et al. (2004) whereas Lee et al. (2000b) reported 39.9 mg protein/kJ was optimum for the same species. The optimum P/E ratio for southern flounder in the present study was 35.4 mg protein/kJ when fed diet 50/10.

In the present study, to increase energy content in the diets, a higher level of carbohydrate (wheat starch) was used in the 45 and 50% protein diets compared to the 55% protein diet. The utilization of carbohydrates for southern flounder was not evaluated in the present study. However, the growth of flounder in the present study was significantly lower for high carbohydrate-based diets (18% and 13% starch for the 45/10 and 45/15 diets, respectively) compared to the other diets containing lower carbohydrates (0–11% starch). This indicated that flounder might not be able to utilize dietary carbohydrate efficiently as an energy source in diets with imbalanced protein and lipid levels. This is consistent with other studies showing that lipid rather than carbohydrate is more efficiently utilized for energy by carnivorous fish (Lee et al. 2002). It is important to determine the utilization of carbohydrate by southern flounder, because imbalance in nonprotein energy sources or levels in the diets might have adverse influences on growth, nutrient utilization, and body lipid deposition (Garling and Wilson 1976).

There is growing awareness of the effects of increased body fat deposition on the quality of fish used for human consumption, as the localization, quantitative importance, and composition of body fat depots may affect their nutritional value, organoleptic properties, processing yield, and storage stability (Cowey 1993). In the present study, the whole body lipid content of southern flounder (1.51–2.31% wet basis) was well within the range of values for similar size juvenile Japanese flounder (1.6–1.9%, Hernandez et al. 2005) when fed a 53% protein and 10% lipid-based diet. Whole body lipid content of southern flounder fed high-lipid diets (2.31–2.64%) was higher than those fed low lipid diets (1.51–1.98%) regardless of dietary protein levels. This is an agreement with what has been observed in other flatfish such as Japanese flounder (Alam et al. 2003; Kim et al. 2004), Atlantic halibut (Aksnes et al. 1996; Helland and Grisdale-Helland 1998), and Senegalese sole fed high and low lipid diets, (Dias et al. 2004).

Finally, growth of southern flounder fed a formulated diet that contained 50% protein and 10% lipid was almost double that of fish fed a commercial diet which contained the same dietary protein and lipid level. This demonstrates that the high quality of protein and lipid sources and a nutritionally balanced formulated diet will be important in developing a commercial diet for maximum growth of southern flounder. The results suggested that a combination of 50% dietary protein and 10% lipid was optimal for the growth performance of juvenile southern flounder fed with a fish meal-based diet reared in a recirculating system.

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Literature Cited

Aksnes A., T. Hjertnes, and J. Opstvedt. 1996. Effect of dietary protein level on growth and carcass composition in Atlantic halibut (*Hippoglossus hippoglossus* L.), Aquaculture 145:225–233.

Alam, M. S. and W. O. Watanabe. 2005. Southern flounder. Aqua feeds: formulation and beyond, 2 (4), Fedware, Florida, USA, 17–19.

Alam, M. S., S. Teshima, S. Koshio, O. Uyan, and M. Ishikawa. 2003. Effects of dietary protein and lipid levels on growth and body composition of juvenile Japanese flounder, *Paralichthys olivaceus* fed intact or crystalline amino acid diets. Journal of Applied Aquaculture 14(3/4):115–132.

Alam, M. S., S. Teshima, D. Yaniharto, S. Koshio, and M. Ishikawa. 2002. Influence of dietary amino acid patterns on growth and body composition of juvenile Japanese flounder, Paralichthys olivaceus, Aquaculture 210:359–369.

Alam, M. S., W. O. Watanabe, and P. M. Carroll. 2008. Dietary protein requirements of juvenile black sea bass *Centropristis striata*. Journal of the World Aquaculture Society 39(5):656–663.

AOAC. 1990. [In] K. Helric, editor. Official methods of analysis of AOAC, 15th edition. Association of Official Analytical Chemists, Inc., Arlington, Texas, USA.

Berge, G. M. and T. Storebakken. 1991. Effect of dietary fat level on weight gain, digestibility and fillet composition of Atlantic halibut. Aquaculture 99:331–338.

Berlinsky, D. L., W. King, T. I. J. Smith, R. D. Hamilton II, J. Holloway Jr., and C. V. Sullivan. 1996. Induced ovulation of southern flounder *Puralichthys lerhosrigma* using gonadotropin releasing hormone analogue implants. Journal of the World Aquaculture Society 27(2):143–152.

Bromley, P. J. 1980. Effect of dietary protein, lipid and energy content on the growth of turbot (*Scophthalmus maximus* L.). Aquaculture 19:359–369.

Bureau, D. P., S. J. Kaushik, and C. Y. Cho. 2002. Bioenergetics. Pages 1–59 *in* J. E Halver and R. W

Hardy, editors. Fish nutrition, 3rd edition. Academic Press, California, USA.

Cho, C. Y. and S. J. Kaushik. 1990. Nutritional energetics in fish: Energy and protein utilization in rainbow trout (*Salmo gairdneri*). World Review of Nutrition, Diet 61:162–172.

Cowey, C. B. 1993. Some effects of nutrition on flesh quality of cultured fish. Pages 227–236 *in* S. J. Kaushik and P. Luquet, editors. Fish nutrition in practice. Proceedings of the IV international symposium fish nutrition and feeding, Les Colloques, 61. INRA Editions, Paris, France.

Daniels, H. V. and W. O. Watanabe. 2003. A practical hatchery manual: production of southern flounder fingerlings. Sea Grant Publication, North Carolina, USA.

Danielssen, D. S. and T. Hjertnes. 1993. Effect of dietary protein levels in diets for turbot (*Scophthalmus maximus*) to market size. Pages 89–96 *in* S. J. Kaushik and P. Luquet, editors. Fish nutrition in practice. Proceedings of the IV international symposium fish nutrition and feeding, Les Colloques, 61. INRA Editions, Paris, France.

De Silver, S. S., R. M. Gunasekera, and K. F. Shim. 1991. Interactions of varying dietary protein and lipid levels in young red tilapia: evidence of protein sparing. Aquaculture 95:305–318.

Dias, J., R. Rueda-Jasso, S. Panserat, L. E. C da Conceic, E. F. Gomes, and M. T. Dinis. 2004. Effect of dietary carbohydrate-to-lipid ratios on growth, lipid deposition and metabolic hepatic enzymes in juvenile Senegalese sole (*Solea senegalensis*. Kaup Aquaculture Research 2004 35:1122–1130.

Gao, Y., J. LV, and Q. Lin. 2005. Effects of protein levels on growth, feed utilization, nitrogen and energy budget in juvenile southern flounder, *Paralichthys lethostigma*. Aquaculture Nutrition 11:427–433.

Garling, D. L. and R. P. Wilson. 1976. The optimum protein-to-energy ratio for channel catfish, *Ictalurus punctatus*. Journal of Nutrition 106:1368–1375.

Gonzalez, S., S. R. Craig, E. McLean, M. H. Schwarz, and G. J. Flick. 2005. Dietary protein requirement for southern flounder, *Paralichthys lethostigma*. Journal of Applied Aquaculture 17(3):37–50.

Helland, S. J. and B. Grisdale-Helland. 1998. Growth, feed utilization and body composition of juvenile Atlantic halibut (*Hippoglossus hippoglossus*) fed diets differing in the ratio between the macronutrients. Aquaculture 166:49–56.

Hernandez, L. H. H., S. Teshima, S. Koshio, M. S. Alam, M. Ishikawa, and H. Tanaka. 2005. Dietary vitamin A requirement of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquaculture Nutrition 11:3–9.

Kikuchi, K. and T. Takeuchi. 2002. Japanese flounder, *Paralichthys olivaceus*. Pages 113–120 *in* C. D. Webster and C. E. Lim, editors. Nutrient requirements and feeding of finfish for aquaculture. CABI publishing, Cambridge, MA, USA.

Kim, K. W., X. J. Wang, and S. C. Bai. 2002. Optimum dietary protein level for maximum growth of juvenile olive flounder *Paralichthys olivaceus* (Temminck and Schlegel). Aquaculture Research 33:673–679.

Kim, K. W., X. Wang, S. M. Choi, G. J. Park, and S. C. Bai. 2004. Evaluation of optimum dietary protein-to-energy ratio in juvenile olive flounder *Paralichthys olivaceus* (Temminck et Schlegel) Aquaculture Research 35:250–255.

Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal number of replications. Biometrics 12:307–310.

Lee, S. M., S. H. Cho, and K. D. Kim. 2000a. Effects of feeding frequency and dietary energy level on growth and body composition of juvenile flounder, *Paralichthys olivaceus* (Temminck & Schlegel). Aquaculture Research 31:917–921.

Lee, S. M., S. H. Cho, and K. D. Kim. 2000b. Effects of dietary protein and energy levels on growth and body composition of juvenile flounder (*Paralichthys olivaceus*). Journal of the World Aquaculture Society 31:306–315.

Lee, S.-M., I. G. Jeon, and J. Y. Lee 2002. Effects of digestible protein and lipid levels in practical feed on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). Aquaculture 211:227–239.

Lee, D. J. and G. B. Putnam. 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. Journal of Nutrition 103:916–922.

Lovell, R. T. 1989. Nutrition and feeding of fish. Van Nostrand Reinhold, New York, New York, USA.

McGoogan, B. B. and D. M. Gatlin. 1999. Dietary manipulations affecting growth and nitrogenous waste production of red drum, *Sciaenops ocellatus* I. Effects of dietary protein and energy levels. Aquaculture 178:333–348.

Nematipour, G. R., M. L. Brown, and D. M. Gatlin III. 1992. Effects of dietary energy: protein ratio on growth characteristics and body composition of hybrid striped bass, *Morone chrysops*' *M. sax-atilis*. Aquaculture 107:359–368.

NRC (National Research Council). 1993. Nutrient requirements fishes. National Academy Press, Washington DC, USA.

Takeuchi, T., Y. Shiina, and T. Watanabe. 1991. Suitable protein and lipid levels in diet for fingerlings of red sea bream *Pagrus major*. Nippon Suisan Gakkaishi 55(2):521–527.

Thoman, E. S., D. A. Davis, and C. R. Arnold. 1999. Evaluation of growout diets with varying protein and energy levels for red drum (*Sciaenops ocellatus*). Aquaculture 176:343–353.

Vergara, J. M., L. Robaina, M. de Izquierdo, and M. la Higuera. 1996. Protein sparing effect of lipids in diets for fingerlings of gilthead sea bream. Fisheries Science 62(4):624–628.

Watanabe, W. O., P. M. Carroll, and H. V. Daniels. 2001. Sustained, natural spawning of southern flounder *Paralichthys lethostigma* under an extended photothermal regime. Journal of the World Aquaculture Society 32:153–166.

Watanabe, W. O., C. A. Woolridge, and H. V. Daniels. 2006. Progress toward year round spawning of southern flounder broodstock by manipulation of photoperiod and temperature. Journal of the World Aquaculture Society 37: 256–272.

Wenner, C. A., W. A. Roumillat, J. E. Moran Jr., M. B. Maddox, L. B. Daniel, and J. W. Smith. 1990. Investigation on the life history and population dynamics of marine recreation fishes in South Carolina: Part I. South Carolina Wildlife and Marine Department Final Report Project F-37. Charleston, South Carolina, USA.