Aquaculture 479 (2017) 780–789

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Effect of winter feeding frequency on growth, survival, and fatty acid metabolism of juvenile bluegill (*Lepomis macrochirus*) and hybrid bluegill (L. *cyanellus* × *L. macrochirus*)

Luke A. Roy[a,*], Steven D. Rawles[b], Anita M. Kelly[a], Carl D. Webster[b], Nathan Stone[a], Alf Haukenes[a]

[a] *Aquaculture/Fisheries Center, University of Arkansas at Pine Bluff, 1200 North University Drive, Pine Bluff, AR 71601, United States*
[b] *Harry K. Dupree Stuttgart National Aquaculture Research Center, PO Box 1050, 2955 Highway 130 East, Stuttgart, AR 72160, United States*

A R T I C L E I N F O

Keywords:
Fatty acids
Winter mortality
Canonical discriminant analysis
Bluegill
Hybrid bluegill

A B S T R A C T

There has been an increasing global demand for large bluegill (*Lepomis macrochirus*) and its hybrid (female *Lepomis cyanellus* × male L. *macrochirus*) to supply the food-fish market. However, production of market-size bluegill requires producers to overwinter them in temperate regions. Winter fish mortality is widely cited by fish farmers in temperate regions of the U.S. as a major factor that decreases profitability and sustainability. We evaluated the effect of three different feeding frequencies on survival, weight loss, and fatty acid composition of two species of Centrarchids held at a constant low temperature (7–9 °C) to simulate winter conditions. Bluegill (1.46 ± 0.06 g) and hybrid bluegill (2.58 ± 0.22 g) were stocked in separate 603-L recirculating systems configured with 9 tanks each (3 replicate tanks/feeding regime) and fed either twice per week, once per week, or once per month for 13 weeks. There were no differences among feeding regimes in final fish weight, survival, weight loss as a percent of initial weight, condition factor, and SGR for either taxon of fish. Survival was high among all treatments (89–98%). Regardless of feeding regime, all fish lost weight. Hybrid bluegill lost less weight (12–17% of initial body weight) than bluegill (18–20%). The concentrations and composition of fatty acids also changed markedly in response to feeding frequency according to canonical discriminant analysis. Fatty acid profiles among initial vs. post-winter bluegills fed at different frequencies were indicative of severe deficiencies in n-6 and n-3 essential fatty acids and preservation of long chain polyunsaturated fatty acids, especially when fish were fed less than twice per week. In conclusion, significant weight loss and reductions in key fatty acids needed for energetic needs were observed in both native and hybrid bluegill, regardless of feeding regime, indicating that the feeding rates examined in this study did not prove beneficial at 7–9 °C in preserving winter robustness.

1. Introduction

There has been an increasing global demand for large bluegill (*Lepomis macrochirus*) and its hybrid (female *Lepomis cyanellus* × male L. *macrochirus*) to supply the food-fish market. This is due to bluegill possessing several characteristics that make them desirable for aquaculture that include rapid growth, acceptance and efficient utilization of prepared diets (Tidwell et al., 1992), and an aggressive nature that makes them attractive to anglers (Brunson and Robinette, 1986). The highest specific growth rate for juvenile bluegill has been reported to occur at 30 °C (optimal temperature range between 26 and 32 °C; Lemke, 1977); however, production of market-size bluegill in the temperate climate of the southeastern United States still requires overwintering.

Winter fish mortality is a phenomenon that has been widely cited by fish farmers in temperate regions of the U.S. as a major factor that decreases profitability and sustainability. As water temperatures decline during the winter months, warmwater fish do not feed as aggressively, or at all. Feeding strategies to reduce mortality and minimize weight loss during this period vary widely in temperate regions of the U.S. Some producers may decrease feeding frequency as temperatures fall toward winter, cease feeding altogether when water temperatures fall below a certain level, or feed intermittently during the winter (Brunson and Robinette, 1986; Webster et al., 1992; Goodgame-Tiu et al., 1994; Rowan and Stone, 1994; McNulty et al., 2000; Nanninga et al., 2011). Currently, there is no generally accepted

* Corresponding author at: School of Fisheries, Aquaculture & Aquatic Sciences, Auburn University, Auburn, AL 36849, United States.
E-mail address: royluke@auburn.edu (L.A. Roy).

http://dx.doi.org/10.1016/j.aquaculture.2017.07.026
Received 7 November 2016; Received in revised form 17 July 2017; Accepted 18 July 2017
Available online 18 July 2017
0044-8486/

protocol practiced industry wide with respect to feeding practices. As feed consumption declines, however, fish weight loss occurs as the metabolism of fish is directly correlated to water temperature, i.e., both routine metabolic rate and metabolic scope, which measure capacity for physiological performance, are significantly depressed in warm water fish with declining temperature (Fontaine et al., 2007). While general culture techniques have made important advances in the production of large bluegill (Tidwell et al., 1994; Hicks et al., 2009; Wang et al., 2009) and nutritional requirements as well as diet formulations have been more clearly defined (Tidwell et al., 1992; Tidwell and Webster, 1993; Hoagland et al., 2003; Twibell et al., 2003; Masagounder et al., 2011; Yang et al., 2016), there are no published data on the effects of different feeding regimens on growth, body composition and health of bluegill. Nevertheless, winter mortality appears to be a universal problem among Centrarchid producers, in spite of a wide range of feeding practices.

One hypothesis suggests that fatty acid composition of overwintering bluegill might impact winter survival through the strong role essential fatty acids (EFAs) play in membrane fluidity, immunity and disease resistance (Tocher and Glencross, 2015). Inadequate levels of EFA can result in growth retardation, fin erosion, myocarditis, increased sensitivity to stress, shock syndrome and death (Castell et al., 1972; Watanabe, 1982). One adaptation to cold temperature is to increase the proportion of long-chain polyunsaturated fatty acids (LC-PUFA) and reduce the proportion of saturated fatty acids in cell membrane phospholipids in order to increase membrane fluidity at lower temperature (Farkas et al., 2001; Tocher, 2003). Any changes in cell membrane fatty acid profiles also alter functions that affect the immune system (Verlhac Trichet, 2010). Reduced availability of LC-PUFA, e.g., EPA and DHA, has been associated with chronic elevation of plasma cortisol, which inhibits immune responses through blocking macrophage cytokine production, thus affecting disease resistance (Montero and Izquierdo, 2010).

Nevertheless, studies on the fatty acid composition of bluegill in regards to environment, diet, or health are extremely rare. Rude et al. (2016) found that LC-PUFA levels were quite substantial but variable in wild caught bluegill from the Illinois River system and ranged from 15 to 30%, depending on the location fish were taken; their data suggest that fatty acid (FA) profiles can infer habitat use and habitat-specific foraging of fishes in large river-floodplain ecosystems. Glass et al. (1974) noted changes in the content of furan fatty acids in liver and testes of bluegill in response to spawning season. Kelly et al. (1958) found no significant change in the PUFA profile of bluegill captured from the wild and those subsequently fed a low-fat or cottonseed oil diet over 60 days, whereas in those fed a menhaden oil diet, the fatty acid composition changed to resemble the composition of the oil. Hence, bluegill may be able to synthesize PUFAs, though not necessarily essential fatty acids, from non-fatty precursors. This is particularly relevant since dietary intake or elongation/desaturation of fatty acids are the two major means of altering PUFA levels in fish (Tocher and Glencross, 2015). If bluegill have limited ability to elongate/desaturate essential PUFA in order to increase membrane fluidity for cold climate, then these essential fatty acids must be ingested (Hazel and Livermore, 1990). In contrast to potential connections between feed intake or feed composition and winter survival of bluegill, Fontaine et al. (2007) suggested that metabolic capacity in warm water fish tends to limit performance at lower temperatures rather than feed composition. Therefore, the objectives of this trial were to evaluate the effect of three different feeding regimes on survival, weight loss and fatty acid composition of two species of Centrarchids held at a constant low temperature to simulate winter conditions.

2. Materials and methods

2.1. Fish and culture system

Bluegill and hybrid bluegill were obtained from J.M. Malone and Son (Lonoke, AR, USA) and transported to the University of Arkansas at Pine Bluff Lonoke Fish Disease Diagnostic Laboratory (Lonoke, AR, USA) in November 2013. Fish were held in separate 150-L holding tanks connected to a larger recirculating system. The fish were held for approximately 6 weeks prior to the commencement of experimental trials to determine that they were disease free and fully acclimated to laboratory conditions. Fish were fed daily ad libitum a 36% protein commercial diet (Delta Western, Indianola, MS, USA) during the holding period. When the trial was initiated, acclimated bluegill (1.46 ± 0.06 g), and hybrid bluegill (2.58 ± 0.22 g) were stocked in separate 603-L recirculating systems as described below and initial samples of whole fish were collected at this time and frozen at − 20 °C until analysis for initial fatty acid profiles.

Each system was configured with 9 tanks (3 replicate tanks per feeding regime) and a sump equipped with a biofilter. Flow rate in each tank was set at 2.4 L min^{-1}. Following stocking of each system (15 fish per tank for a total of 135 fish per system), temperature was reduced from ambient (22 °C) to a target temperature of 8 °C over a period of 7 days. Water temperatures in the culture systems were maintained by using drop-in chillers (0.19 KW) with digital temperature controllers (TradeWind Chillers, Escondido, CA, USA). Water chillers were the same model, and water volumes were nearly identical in the two experimental systems. However, the capacity of individual chillers to maintain target temperatures varied somewhat resulting in slight temperatures differences among systems. Temperature was monitored twice daily (morning and evening) and adjusted as needed. Mean water temperatures were maintained between 7.0 and 9.2 °C in all trials.

Total ammonia nitrogen, dissolved oxygen and pH remained within acceptable limits. Water quality (mean ± standard deviation) was acceptable for the culture of both species. In the native bluegill trial dissolved oxygen (9.41 ± 0.80 mg L^{-1}), temperature (7.00 ± 0.60 °C), pH (8.10 ± 0.20), total ammonia nitrogen (0.31 ± 0.11 mg L^{-1}), and nitrite nitrogen (0.00 ± 0.00 mg L^{-1}) remained within acceptable limits. In the hybrid bluegill trial dissolved oxygen (9.09 ± 0.83 mg L^{-1}), temperature (8.00 ± 1.25 °C), pH (8.14 ± 0.21), total ammonia nitrogen (0.32 ± 0.13 mg L^{-1}) also remained within acceptable limits. Nitrite nitrogen (0.31 ± 0.34 mg L^{-1}) was slightly high but did not appear to negatively impact the fish. There was a 3-week spike in nitrite-nitrogen (1.5 mg L^{-1}) in the hybrid bluegill system, however no mortalities occurred as a result. When the spike occurred, sodium chloride was added (0.25 g L^{-1}) to the system to raise chloride levels.

2.2. Feeding regimes and sampling

Bluegill and hybrid bluegill were fed ad libitum the same commercial diet (Table 1) offered during the experimental period using three different feeding regimes. Feeding regimes consisted of either twice per week (2 ×/wk), once per week (1 ×/wk), or once per month (1 ×/mo). These feeding regimes were determined based on the range of current winter feeding practices of commercial bluegill and hybrid bluegill farmers in Arkansas. Both taxa consumed feed during feeding events throughout the trial, albeit very little in some cases. Fish were given 5–10 min to feed. If feeding ceased no further feed was offered on that day. Excess feed was not removed from the tanks. Following 13 weeks of culture at low temperature, the trials were terminated to assess weight loss, survival, specific growth rate and Fulton condition factor. Weight loss, specific growth rate (SGR) and Fulton condition factor (K) were calculated as follows:

$$\text{Weight loss (\%)} = 100\,(W_f - W_i)/W_i,$$