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SEASONAL VARIATION OF REPRODUCTION AND FATTENING IN GULF KILLIFISH FROM BRACKISH MARICULTURE PONDS[1]

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ABSTRACT: Seasonal changes in gonadal indices, plasma androgens in males, plasma estrogens and egg production in females, and body fat content in both sexes were monitored in gulf killifish, *Fundulus grandis*, reared in mariculture ponds. Changes in plasma androgens (males) and estrogens (females) were positively correlated with changes in gonadosomatic indices (GSI) of both sexes during most of the year. Seasonal variation in the GSIs of a nearby natural marsh population were similar, though not identical, to that of the pond-raised population. However several distinct differences were noted between these two populations: 1) spawning was prolonged in the mariculture population as compared with the wild population; 2) body fat content of the mariculture fish was significantly greater than wild fish during all seasons of the year; 3) a significant negative correlation between the seasonal cycles of body fattening and reproduction was observed in the mariculture fish but not in the wild fish. These data indicate that the mariculture environment and resultant high levels of body fattening supported prolonged spawning in this species.

Seasonal cycles of reproduction (de Vlaming, 1974) and fattening (Shul'man, 1974) are characteristic of temperate zone fish. In general, these cycles are inversely related (Nikolskii, 1963; de Vlaming, 1971; Shul'man, 1974) due to the utilization by teleosts of fat reserves as the primary energy source for generative purposes, including reproduction (Shul'man, 1974). Food availability is considered the ultimate controlling factor for both reproduction (de Vlaming, 1974) and fattening (Shul'man, 1974), with temperature and photoperiod acting as proximate cues for synchronization of these cycles (de Vlaming, 1974).

Where food supply is relatively constant, as can be the case in lower latitudes, reproduction (Nikolskii, 1963) and fattening (Shul'man, 1974) are less seasonally variable than in temperate zone fish. In a recent survey of seasonal reproduction and lipid content of several subtropical cyprinodontids from the Gulf coast, including the gulf killifish, *Fundulus grandis*, de Vlaming *et al.* (1978) concluded that these fish differed from fish of higher latitudes in several ways: subtropical cyprinodontids had protracted breeding seasons, limited seasonal variability of fat reserves, and no detectable relationship between reproduction and fattening. An abundant year-round food supply was suggested to be the reason for the lack of a significant negative correlation between reproduction and fattening typically observed in temperate zone fish.

The present investigation was conducted on *F. grandis* reared in brackish-water mariculture ponds. This study, undertaken to investigate the feasibility

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of raising these fish for the bait industry, provided an opportunity to determine how controlled high food availability throughout the year affects reproduction and fat storage in a southern temperate-subtropical environment.

Seasonal variations in gonadal growth, plasma gonadal steroids (androgens and estrogens), egg production and body fat content were measured in this cultured population of *F. grandis* and compared to reproductive and fattening indices of fish sampled from a local salt marsh.

METHODS

All ponds used for holding, spawning, hatching and grow-out studies were 0.8ha in mean surface area and 1.0m in mean depth, located at the Claude Peteet Mariculture Center (CPMC) on the southeast side of Mobile Bay, Baldwin County, Alabama. Fish were fed a commercial floating feed (Purina Trout Chow) twice daily (4-5% body weight/day). Chicken manure (284 kg/ha) was occasionally added to promote natural pond productivity. Further details of the pond conditions are described by Tatum *et al.* (1979).

In February 1978, brood fish were stocked from a population reared at CPMC since 1975 into two spawning ponds (500 males & 500 females per pond). Ten male and female brood fish were sacrificed on the following dates: 1) 20 February prior to stocking spawning ponds, 2) 20 March prior to setting spawning mats for egg counts, 3) 16 May when offspring were harvested from spawning and hatching ponds, 4) 18 August during a late summer spawning study, 5) 28 October prior to transfer to holding ponds(overwintering), and 6) January 1979 prior to restocking spawning ponds. Body lengths and weights were recorded, blood plasma collected and frozen for later analysis and gonads weighed and preserved in buffered 20% formalin; gonadosomatic index (GSI) was calculated as gonad weight as a percent of body weight. After removal of the gonads, the fish were dessicated to a constant weight. Dried carcasses were then weighed, cut into small sections and lipids extracted for 48 hours with petroleum ether; longer extraction periods did not yield significantly greater amounts of lipids. Tissues were washed three times with fresh petroleum ether and the ether extract evaporated at 40° C. Body fat was expressed as a percent of dried body weight.

Spawning activity was assessed from estimates of eggs deposited on spawning mats (Tatum *et al.*, 1979). Estimates were made daily from 21 March through 4 April by counting eggs in a random number of mesh squares for each mat. Mats with eggs then were transferred by hand to hatching ponds. On 16 May, hatching ponds and brood ponds were drained to collect offspring for distribution to grow-out ponds.

On 31 July, spawning mats were replaced in brood stock ponds to assess late summer spawning. In addition, on 18 August, spawning mats were placed in grow-out ponds to assess spawning activity of young-of-the-year. Egg production was estimated weekly for each pond.

For comparative purposes gulf killifish were collected at approximate monthly intervals during the study period from a salt marsh at the Point aux Pins Marsh Lab (PPML: Marine Environmental Sciences Consortium of Alabama), located west of Mobile Bay on the Mississippi Sound, near Bayou La Batre in Mobile County, Alabama. Fish were collected in minnow traps and by seining in shallow (less than 1.0m deep) inlets. Body lengths and weights were recorded, gonads weighed and preserved and fat extracted. Body sizes of sample populations were similar (pond males: 9.92g-

63.55g; pond females: 10.61g-54.17g; marsh males; 4.61g-55.07g; marsh females: 5.15g-60.92g). Spawning activity, egg production and plasma steroids were not determined for the marsh population.

Plasma Steroid Assay

Androgens (testosterone and 11-ketestosterone) in male plasma and estrogens (estradiol and estrone) in female plasma were extracted and quantified by radioimmunoassay as outlined in Dindo and MacGregor (1981). Because plasma extracts were not chromatographically purified for testosterone or estradiol-17β, steriod concentrations were expressed as androgens (males) and estrogens (females). This procedure has provided a good indicator of reproductive condition when applied to other teleosts (Dindo and MacGregor, 1981; MacGregor *et al.*, 1981).

Accuracy of the assays with *Fundulus* plasma was determined by measurement of aliquots of different volumes from a sample pool to verify a linear response. Buffer blanks and water blanks repeatedly gave readings equal to or less than 5 pg/100 μl. Plasma aliquots reading less than 10 pg/100 μl were considered non-detectable. The data were tested for satistical differences by analysis of variance and modified Duncan's New Multiple Range test (Steel and Torrie, 1960).

RESULTS

Significant seasonal variations in gonadosomatic indices (GSI) and plasma gonadal steriods were observed in both sexes of *F grandis* sampled from the mariculture ponds. Among males a rapid increase in GSI and plasma androgens occurred between February and March (Fig. 1). Gonadosomatic indices of males were significantly lower ($P < 0.01$) in May (0.44 ± 0.05%) than in March (0.77 ± 0.06%). Lowest values were observed in August (0.26 ± 0.05%) and November (0.25 ± 0.04%). By late January of the following year (1979) GSIs (0.49 ± 0.03%) were not significantly different than those of the previous February. Spermatogenesis was evident in all samples, though quantities of mature sperm were noticeably less in October samples than those from other months.

Plasma androgens in males were high in March (3.20 ± 0.50 ng/ml) and declined rapidly through May (1.35 ± 0.30 ng/ml) and August (0.69 ± 0.18 ng/ml). A significant linear correlation $r = 0.79$, $P < 0.01$) was calculated between plasma androgens and testicular GSIs of *F. grandis* from the mariculture ponds. However, androgens remained low in January as testicular indices were first beginning to increase (Fig. 1).

In female killifish from the mariculture ponds, GSIs were highest in March (7.5 ± 0.5%) and May (8.7 ± 1.2%) while ovaries contained many ripe ova and follicles in all stages of development (Fig. 2). Female GSIs were lowest (0.52 ± 0.20%) in late October, with no ripe or maturing follicles present.

Plasma estrogens in female *F. grandis* were highest in March (2.40 ±

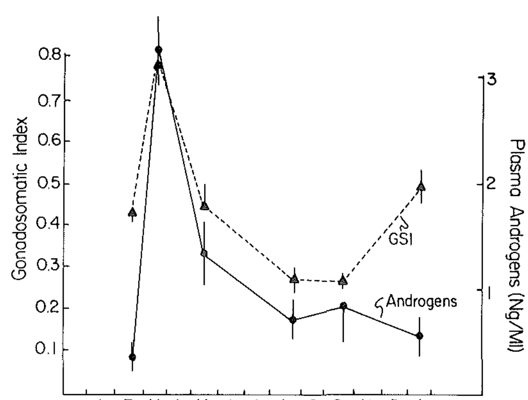

Figure 1. Seasonal variation in gonadosomatic index and serum androgens of male *Fundulus grandis* reared in brackish mariculture ponds. Each point represents the mean ± S.E. for 10 fish.

1.00 ng/ml) and remained elevated through August (1.50 ± 0.40 ng/ml). Estrogens were reduced in October (0.57 ± 0.39 ng/ml) and remained low through January (.085 ± 0.36 ng/ml). A significant linear correlation ($r = 0.53$, $P < 0.01$) was calculated for the seasonal cycles of plasma estrogens and ovarian GSIs.

The rapid rise in GSIs and plasma gonadal steroids immediately preceded the onset of spawning at the Mariculture Center, as indicated by the dates when eggs were first deposited on spawning mats (Table 1). The onset of spawning was coincident with a rise in pond temperatures from 10° to 20° C during March. A marked increase in recorded daily egg production in all three ponds occurred between 1 April and 4 April (Table 1). Spawning on mats continued at this elevated level through April and May (not shown). After transfer of brood stock to two other ponds (B-3 and B-4) on 7 July, egg production estimates indicated continued spawning activity in August, September and October (Table 2). Average temperature in the ponds at these times were 31° ± 5° (Aug.), 27° ± 3° (Sep.) and 23°±5° (early Oct.). Egg production during this autumn period was significantly reduced compared to

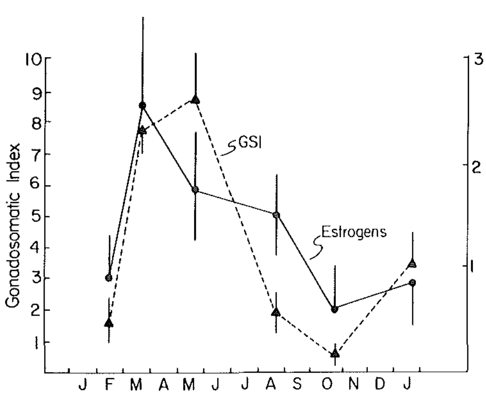

Figure 2. Seasonal variation in gonadosomatic index and serum estrogens of female *Fundulus grandis* reared in brackish mariculture ponds. Each point represents the mean ± S.E. for 10 fish.

Table 1. Eggs deposited on spawning mats during March and April 1978 in spawning ponds by *F. grandis.*

	Eggs per day per pond[1]		
Date	Pond C-3	Pond C-5	Pond B-7
March			
21	0	0	0
22	40	10	10
23	92	40	210
24	740	590	165
25	340	350	560
26	300	310	no count
27	685	510	280
28	680	395	330
29	680	385	380
30	915	3800	980
31	900	725	325
April			
1	2225	920	475
2	1330	1425	850
3	2870	2575	1285
4	2875	3225	1335

[1]Eight spawning mats per pond; 500 pairs of *F. grandis* per pond.

the spring spawn. No eggs were deposited on spawning mats after 20 October, at which time histological examination of ovaries indicated cessation of further fillicle maturation.

Young of the current year's hatch also were monitored from July through October (Table 2). Spawned eggs were first observed on 19 August (110-125 eggs/day/pond). A peak in first-year spawning activity occurred from 8 September to 22 September (Pond A7: 831 ± 74; Pond B-9: 666 ± 153 eggs/day/pond). Thereafter egg production rapidly declined, with no eggs deposited after 20 October.

A similar and significant seasonal variation in GSIs ($P < 0.01$) was observed in both sexes of *F. grandis* collected from the marsh at Point aux Pins (Fig. 3). Peak values of male and female GSIs in March and April were not significantly different from mariculture fish. However, males from the marsh did attain a lower average GSI (0.10 ± 0.02%) in the fall than the pond-raised killifish, though testicular histological appearance was not significantly different. Female GSIs of the

Table 2. Eggs deposited on spawning mats from 28 July 1978 to 28 October 1978 by adult and juvenile *F. grandis.*

	Eggs per day per pond[1] Adult Fish		Eggs per day per pond[2] Juvenile Fish	
Date	B-3	B-4	A-7	B-9
28-31 July	408	158	0	0
1-4 August	146	100	0	0
5-11	353	304	0	0
12-18	540	385	0	0
19-25	322	243	125	110
26-31	340	406	75	125
1-8 September	969	528	875	750
7-15	494	303	625	875
16-22	367	318	976	375
23-30	182	442	850	180
1-6 October	268	414	525	75
7-13	163	45	375	0
14-20	76	25	180	125
20-28	0	0	0	0

[1]Eight spawning mats per pond; 500 pairs of fish per pond.
[2]Eight spawning mats per pond; 1500 pairs of fish per pond.

marsh population rapidly decreased from March (9.78 ± 0.99%) through May (4.13 ± 0.48%) and September (0.49 ± 0.06%). No ripe or developing follicles were evident in the latter fish although spawning continued in the mariculture population. Temperatures in the marsh were comparable to those in the ponds, averaging 30° (Aug.) and 27° (Sept.).

Body fat indices varied significantly during the year in adult fish sampled from the mariculture ponds (P <0.05). Fat content did not differ significantly between sexes; therefore both male and female values were averaged for each location (Fig. 4). Annual fluctuations of fat indices were greater in mariculture fish (more than two fold difference between minimum and maximum) than wild fish (less than one-half fold difference between minimum and maximum). Body lipid indices in pond fish were also greater than in wild fish at all sample periods. Furthermore, the months of minimum and maximum fat content differed between the sample sites. Body lipid content was greatest for mariculture fish in August and October and least in February and May. In the wild killifish, body lipid content did not vary significantly, but tended to be higher in May and November than in February, August and January. Comparisons of monthly GSIs and fat indices of mariculture fish yielded a significant negative correlation (r = −0.616, P <0.01). In contrast, no significant correlation was observed between GSIs and fat content of wild killifish.

DISCUSSION

Seasonal variations in gonadal development, plasma gonadal steroids,

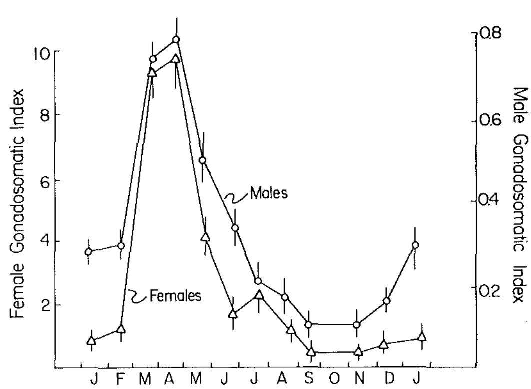

Figure 3. Seasonal variation in gonadosomatic indexes of male and female *Fundulus grandis* collected from a salt marsh near Mobile Bay. Values are means ± S.E.

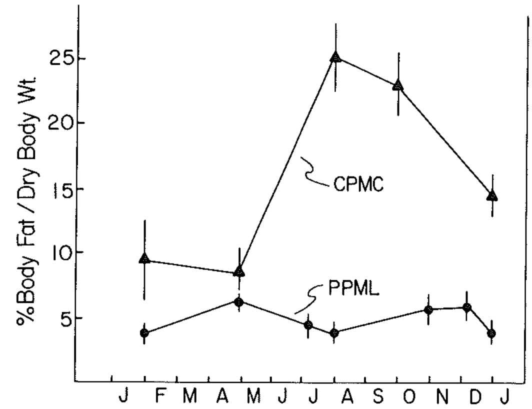

Figure 4. Seasonal variation in body fat as percent of dry body weight of *Fundulus grandis* from mariculture ponds (CPMC) or a local salt marsh (PPML). Values are means ± S.E.

and body fat content were very distinct in gulf killifish reared in brackish water mariculture ponds. Prior to the initiation of spring spawning, a rapid increase in plasma gonadal steriods occurred with increasing gonadosomatic indices in both sexes of killifish. This observation is similar to other seasonal correlations of gonadal maturation and increased plasma or serum androgens as reported for male sockeye salmon, *Oncorhynchus nerka* (Schmidt and Idler, 1962), Atlantic salmon, *Salmo salar* (Idler *et al.*, 1971), plaice, *Pleuronectes platessa* (Wingfield and Grimm, 1977) and striped mullet, *Mugil cephalus* (Dindo and MacGregor, 1981) and for estrogens in female rainbow trout, *Salmo gairdneri* (Lambert *et al.*, 1978) plaice (Wingfield and Grimm, 1977), goldfish, *Carassius auratus* (Schreck and Hopwood, 1974), striped mullet (Dindo and MacGregor, 1981) and bluefish, *Pomatomus saltatrix* (MacGregor *et al.*, 1981).

However, in both sexes of killifish the initial winter (December — February) increases in GSIs observed during the early stages of gonadal development were not accompanied by a significant change in either plasma estrogens or androgens. This suggests that plasma estrogens and androgens are not associated with the early stages of gonadal recrudescence, an idea supported by other studies on rainbow trout (Boheman and Lambert, 1981), plaice (Wingfield and Grimm, 1977), stiped mullet (Dindo and MacGregor, 1981) and bluefish (MacGregor *et al.*, 1981).

The seasonal changes in GSIs of wild and pond fish were very similar. Rapid gonadal maturation occurred during pond and marsh temperature changes from 10° to 20°C in March. Differences in the timing of seasonal maturation when comparing this and other studies on *Fundulus* sp. may be explained by differences in local thermal changes. In *F. similis* from Corpus Christi, TX, the prespawning rise in GSIs occurred in January (de Vlaming *et. al.*, 1978); in *F. grandis* from Grand Isle, LA, GSIs also peaked in January (Fivizzani, 1977); in *F. heteroclitus* from New Haven, CT, GSIs peaked in May (Balbontin *et. al.*, 1978) while in a Delaware Bay study ripe females were first noted on 30 March (Taylor *et al.*, 1979). Common to all these reports were water temperatures approaching 20°C. However, day lengths were significantly different among these studies. These data indicate that seasonal onset of spawning in *Fundulus* appears to be stimulated by temperatures approaching 20°C and support a hypothesis of thermal control of reproduction in cyprinodontids as presented by de Vlaming (1974).

Body fat content in the marsh population (body fat index, ranges: 2.0%-10% dry body weight) was similar to those reported for other natural populations of *F. grandis* (Fivizanni, 1977; de Vlaming *et al.*, 1978). However, body fat in the pond-raised killifish (9.5% to 25.0% dry weight) greatly exceeded these values. These data are consistent with observations on trout indicating that hatchery fish have higher fat content than wild trout (Phillips, 1969). Phillips (1969) suggested the primary cause for this comparative fattening to be the higher caloric content of hatchery food versus natural foods. Higher caloric content, less energy expenditures due to the confined environment, and a constant food supply are probably all factors favoring the deposition of large amounts of body fat in pond-raised killifish.

Maximal fat deposition in pond-raised fish occurred during the summer months. Body fat was subsequently reduced during the winter prespawning period. Even though food was available, very little feeding activity was observed during the winter months. Thus pronounced seasonal variations in body fat content occurred even in the presence of constant food availability. This observation conflicts somewhat with the popular concept that fattening cycles in fish are closely related to seasonal cycles of food availability (Nikolskii, 1963; Shul'man, 1974). It appears that behavioral or physiological inhibition of feeding during the winter months, probably due to a temperature effect, limited the energy intake needed for normal metabolism and reproductive development. A decrease in fat stores indicated that fat was being utilized for these energy purposes, principally for reproductive development, since metabolic needs during the winter months would be reduced by the quiescent habit of the killifish in cold temperatures.

Shul'man (1974) has indicated that fat is the major energy source for reproductive development in fish. Temperate zone fish typically exhibit an inverse relationship between seasonal cycles of fattening and reproduction (Shul'man, 1974; de Vlaming *et al.*, 1975, 1977), a natural consequence of fat stores being utilized and depleted for reproductive purposes when available energy is a limiting factor. Tropical fish experience less seasonal variation in environmental parameters, so in consequence demonstrate less seasonal variation in physiological parameters. These fish typically lack an obvious relationship between fattening and reproduction (Shul'man, 1974).

As a result of the summer build-up and winter depletion of fat stores in the pond-raised killifish, this population exhibited a negative correlation of body fat content with gonadosomatic indices characteristic of wild temperate zone fish. In contrast, this relationship was not apparent in the marsh population. No significant variation in body fat content was observed in these killifish, a situation similar to that of tropical fish. de Vlaming *et al.* (1978), although finding limited seasonal variation in the lipid content of various cyprinodontid species (including *F., grandis*) along the Gulf coast, were also unable to establish a relationship between fattening and reproduction in natural populations of these killifish. These authors suggested the lack of such a relationship could in part be due to the more constant year-round food availability in sub-tropical regions, such as the Gulf coast, when compared with higher latitudes. The data from the present study do not support such a hypothesis, since pond-raised fish had an excess of food available year-round and exhibited a distinct inverse relationship between reproduction and fattening. The absence of a relationship between fattening and reproduction is more likely due to an effect of seasonal limitations in the amount and caloric content of available foods when conditions (i.e., energy requirements) are otherwise favorable for fat deposition. More information on the seasonal cyclicity of natural food supplies and metabolic energy demands would be needed to confirm this hypothesis.

Assuming that fat is indeed the primary energy source for reproductive purposes in fish, de Vlaming *et al.*, (1978) suggested that the extended breeding seasons of some cyprinodontids are related to the comparatively large fat stores of these fish. Similarly, increases in food availablity have been reported to hasten seasonal reproductive maturation in a number of fishes (Gross, 1949; Cushing and Burd, 1956). In the present study, pond-raised mariculture fish with both large fat stores and high food availability began spawning in mid-to-late March 1978, as evidenced by the appearance of eggs in spawning mats (Tables 1 and 2). Spawning in this population continued into October, as did spawning by reproductively mature young-of-the-year. Spawning likewise began in the marsh population in March, but ceased by September as evidenced by the histological condition of gonadal samples. Therefore, increased fat stores and food availability had no observable effect upon the onset of spawning. However, the spawning season of the mariculture population was extended as compared with the marsh population, although other environmental factors, particularly temperature and photo-period, were not significantly different between the populations. *Fundulus* characteristically have protracted breeding seasons with numerous spawning episodes rather than a single or few seasonal spawns. Thus, a major source of energy utilized by these fish may actually be the immediate food supply. The pond-raised fish presumably had more energy available in the summer and fall months than did their marsh fish, as indicated by the extended breeding season and extensive fat deposition. Unfortunately, direct comparison of energy intake versus expenditures was not possible from these data.

An alternate explanation of these data may be that the reproductive potential of the mariculture population was greater than the natural population from the onset of the breeding season. The larger initial gonadal size of the pond-raised fish, especially the females, during the pre-spawning preparatory period of November to January reflects a greater proliferation of germinal tissue in these fish versus wild fish. The accompanying depletion of fat stores in the former population indicates that this preparatory period of reproductive development in *F. grandis* was augmented by the large over-wintering fat reserves accumulated by the pond-raised fish in response to the favorable mariculture environment.

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